REPORT

Health Effects Associated With Short-Term Exposure To Low Levels of Hydrogen Sulphide (H_2S)

- A Technical Review -

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HEALTH AND WELLNESS

Health Surveillance

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HEALTH EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO LOW LEVELS OF HYDROGEN SULPHIDE (H_2S)
- A TECHNICAL REVIEW -

Prepared for:

Alberta Health and Wellness
Health Surveillance
Health Strategies Division

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EXECUTIVE SUMMARY

Health Effects Associated with Short-term Exposure to Low levels of Hydrogen Sulphide (H_2S) – A Technical Review[1]

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations - Final Report.* The report contained a series of recommendations aimed, in part, to increase awareness of sour gas and its potential impacts on public health.

In response to *Recommendation 9*[2], Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). Cantox Environmental Inc. was retained by AH&W to perform the review. The review focused on the health effects information on short-term exposures to H_2S at concentrations in the range of 0 to 100 ppm. A principal aim of the work was to update the conclusions reached earlier by the Ad Hoc Committee on H_2S Toxicity (Alberta Health, 1988).

The review was performed under the auspices of an Expert Panel, with members drawn from Alberta Environment, regional health authorities, industry, and other stakeholders. Terms of Reference for the work were developed over a six-month period, after which a detailed and comprehensive review of the available scientific literature was completed. The Terms of Reference specified that the review was to focus on the health effects of short-term exposures to low levels of H_2S on normal, healthy individuals. Thus, issues surrounding "hypersusceptible" individuals were not directly addressed. Similarly, health effects that might occur secondary to the odour of H_2S were not specifically addressed.

A set of eligibility criteria was developed to properly focus the review on studies found in the published scientific literature that satisfied the Terms of Reference. The eligibility criteria allowed for the systematic selection of studies based on both technical and practical considerations. Technical demands included the need for the studies to have tested for health effects within the concentration range of interest (*i.e.*, 0 to 100 ppm) for a prescribed duration (*i.e.*, up to 30 days) using the most appropriate mode of exposure (*i.e.*, inhalation). Practical requirements included the need for the studies to have been published in the English language in peer-reviewed scientific publications that were readily available.

A set of 45 studies met the eligibility criteria for review. The studies included clinical studies involving controlled exposures of volunteer human subjects, non-clinical investigations involving controlled exposures of test animals, and case-control studies involving evaluation of individuals exposed to H_2S as a result of accidental releases. Non-clinical studies dominated the set, with close to 75 percent of the 45 studies involving animal testing.

The review included a critical assessment of the overall technical quality of each study based on consideration of experimental design, conduct and reporting, as well as grading of the level of confidence that could be assigned to the study findings and conclusions. A pre-defined set of "quality" criteria was developed to ensure that assessment and grading were performed objectively and consistently.

The assessment of technical quality revealed a number of common weaknesses in experimental design, execution and reporting. Only 15 percent of the studies achieved a "high" confidence index ranking. Close to 50 percent of the studies were assigned a "low" confidence index ranking. Common weaknesses included failure to follow conventional testing protocols, failure to follow Good Laboratory Practice (GLP) guidelines, use of a single exposure concentration only, use of a single sex only, use of too few test subjects, lack of routine measures of toxicity, and inadequate descriptions of exposure conditions. In addition, a large number of the studies were directed at understanding of the mechanism of action of H_2S as opposed to identifying health effects *per se*.

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[1] Report prepared by Donald B. Davies, Ph.D., DABT and Susan E. Haggarty, B.Sc. (Cantox Environmental Inc.) for Alberta Health and Wellness, July 2002.

[2] Recommendation 9 reads as follows: The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed as soon as practical due to its urgency. (Alberta Health and Wellness has committed to lead the review for H_2S).

Apart from gauging the technical quality of each study, the review included critical assessment of the toxicological significance and clinical relevance of the study findings. The findings were summarized and a weight-of-evidence approach was used to assess the meaning and relevance of the overall health effects information. Emphasis was given to studies assigned a "moderate" or "high" confidence index ranking for this purpose. Clinical and non-clinical findings were differentiated.

The review made no attempt to extrapolate the results from the animal testing to the human condition.

The following conclusions emerged from the review:

A. Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations could not be firmly established.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

B. Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting. The toxicological significance and clinical relevance of the responses was not readily apparent.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects remained unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. The signs and symptoms that did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al.*, 1940). In a second older study conducted by Mitchell and Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

No indications of significant effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed before, during and after gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species were not found.

TECHNICAL SUMMARY

Health Effects Associated with Short-term Exposure to Low levels of Hydrogen Sulphide (H_2S) – A Technical Review[3]

Background

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations - Final Report.* The report contained a series of recommendations aimed at increasing awareness of sour gas and its potential impacts on public health, improving the regulatory system surrounding sour gas, and fostering better communication between all affected stakeholders on matters relating to sour gas.

In response to *Recommendation 9*[4], Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). In keeping with the mandate of the Provincial Advisory Committee, the focus of the review was to be on the health effects of "acute" or short-term exposure to H_2S. By way of direction, the Advisory Committee referred to the *"table of effects of H_2S"* that had been prepared by AHEW on the basis of conclusions reached by the Ad Hoc Committee on H_2S Toxicity following an earlier review of the literature (Alberta Health, 1988). The Provincial Advisory Committee urged that a revised table be developed as soon as possible.

In fulfilling its commitment, Alberta Health and Wellness retained Cantox Environmental Inc. ("the consultant") to perform the review. Consistent with the direction provided in *Recommendation 9*[4], the review was conducted under the auspices of an Expert Panel, with members drawn from Alberta Environment, regional health authorities, industry, and other stakeholders. The Expert Panel was charged with:

- Providing advice and direction in relation to the overall scope and nature of the work.
- Assisting in the development of the exact Terms of Reference for the work.
- Helping to identify scientific literature pertaining to the health effects of H_2S.
- Assisting in the development of objective criteria for assessing the technical quality of the scientific literature.
- Providing technical advice to assist in the grading and interpretation of the scientific literature.
- Critiquing progress and draft reports prepared by the consultant.

Terms of Reference

Terms of Reference for the work were developed over a six-month period, after which a detailed and comprehensive review of the available scientific literature was completed. The final Terms of Reference were:

- The review was to focus on the health effects following short-term exposure. The term *"short-term"* was to include exposures of both an acute and sub-acute variety, to capture exposures lasting a few hours to a few days.
- The review was to focus on health effects following 'low dose' exposures. The term *"low dose"* was to include concentrations of H_2S up to and including 100 ppm.
- The review was to focus on health effects *per se*. Although a formal definition of *"health effects"* was not adopted by the Expert Panel,

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[3] Report prepared by Donald B. Davies, Ph.D., DABT and Susan E. Haggarty, B.Sc. (Cantox Environmental Inc.) for Alberta Health and Wellness, June 2002.

[4] Recommendation 9 reads as follows: *The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed as soon as practical due to its urgency. (Alberta Health and Wellness has committed to lead the review for H_2S).*

the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*

- The review was to give special consideration to the potential health effects of H_2S on the developing fetus in light of the potential increased sensitivity of this life-stage to the harmful effects of chemicals.
- The review was to focus on scientific studies involving exposures to H_2S via inhalation in order to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (*e.g.*, oral, dermal, injection) were to be excluded from review.
- The review was to be limited to information found in peer-reviewed scientific publications.
- The review was to focus on full-length, primary scientific papers describing original work, rather than on review articles or abstracts.
- The review was to include consideration of information from clinical investigations involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory, and "population" studies involving exposures following routine or accidental releases of H_2S into the environment.
- The review was to include a critical assessment of the technical quality of each scientific paper based on consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

It was determined that the review of the health effects information would not specifically address the potential physiological or psychological responses that might be elicited by unpleasant odours, such as the characteristic "rotten egg" smell of H_2S. However, at the same time, it was determined that the review should carefully document studies for which odour complaints were recorded, including descriptions of any signs and symptoms observed that may have been related to the odour of H_2S.

Similarly, the Expert Panel agreed that emphasis would be directed at assessing the effects of exposure to H_2S on normal populations, but information which emerged during the review which related to the issue of "hypersusceptibility" also would be captured. The need to consider such information respected the fact that a significant portion of the general population may have pre-existing health problems, such as cardiovascular or respiratory disease, that may increase susceptibility to H_2S.

Methods

A comprehensive search of the scientific literature was performed to identify studies that complied with the Terms of Reference. A staged search strategy was employed, with primary reliance placed on electronic databases accessed through the DIALOG Information Retrieval Service. Secondary search methods were used to confirm the completeness and adequacy of the DIALOG search, and included manual searching of literature citations found in review articles and personal communication with the members of the Expert Panel and other specialists in the field. The various papers identified through the search were retrieved and then reviewed against a set of pre-determined "eligibility criteria" that were fashioned around the Terms of Reference for the work. The eligibility criteria required that the exposure conditions used in the studies be adequately documented and consistent with the Terms of Reference vis-à-vis exposure concentration tested (*i.e.*, 0 to 100 ppm), exposure duration (*i.e.*, up to 30 days), mode of exposure (*i.e.*, inhalation) and other applicable qualifiers.

A set of 45 studies met the eligibility criteria for review. The studies included clinical studies involving controlled exposures of volunteer human subjects, non-clinical investigations involving controlled exposures of test animals, and case-control studies involving evaluation of individuals exposed to H_2S as a result of accidental releases. Non-clinical studies dominated the set (75%). Very few clinical studies (n=8) or case-control studies (n=4) satisfying the eligibility criteria were identified.

Each of the 45 studies was subjected to independent review by two members of the consultant's team. The review proceeded in two stages. The first stage included critical assessment of the overall technical quality of the study based on consideration of experimental design, conduct and reporting, as well as grading of the level of confidence that could be assigned to the study findings and conclusions. A pre-defined set of "quality" criteria was developed to ensure that assessment and grading were performed objectively and consistently. The quality criteria were based largely on the recommendations of leading scientific authorities for the proper design, execution and reporting of health effects studies. The authorities included the Organization for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (USEPA). Each study was graded or ranked (*e.g.*, high, moderate, low) according to how well the quality criteria were satisfied.

The second stage of the review involved critical interpretation of the findings from each study, and assessment of the toxicological significance and clinical relevance of any observed responses. The collective findings from all studies were then summarized and a weight-of-evidence approach was used to assess the meaning and relevance of the overall health effects information.

The review of the 45 studies that met the eligibility criteria proceeded with a clear understanding of the need for objectivity, consistency and scientific rigor. Emphasis was assigned to those studies that achieved a "confidence index ranking" of moderate or high. Studies assigned a low ranking received less attention, but were included for completeness. The case-control studies consistently received "low" confidence index rankings owing to inadequate exposure characterization, possible subject bias, inconsistent reporting of findings and generalized weaknesses in experimental design, conduct and reporting. Accordingly, very little attention was given to the findings and conclusions from these studies.

Findings

The assessment of technical quality revealed a number of weaknesses in experimental design, conduct and/or reporting. Only 15 percent of the studies achieved a "high" confidence index ranking. Close to 50 percent of the studies were assigned a "low" rating. The common weaknesses included:

- Failure to follow conventional testing protocols. (Only 20 percent of the studies followed protocols that matched or approached those recommended by leading scientific authorities).
- Failure to follow Good Laboratory Practice (GLP) guidelines. (Reference to GLP was found in one study only).
- Use of a single exposure concentration only. (Close to 50 percent of the studies involved testing with a single concentration of H_2S, thereby precluding assessment of the dose-responsiveness of any observed health effects, and preventing any meaningful conclusions as to whether or not any responses were treatment related).
- Use of a single sex only. (Forty percent of the studies involved testing with one gender, thereby limiting the ease of extrapolation of any findings to the general population).
- Use of too few test subjects. (Many studies used only limited numbers of test animals or human subjects, thereby rendering interpretation of the significance of any observed health effects difficult).
- Lack of routine measures of toxicity. (Most studies failed to include measurement of routine toxicity endpoints, such as signs and symptoms, body weights and pathology. The lack of this information added to the difficulty in interpreting the biological significance of any observed changes).

- Inadequate descriptions of exposure conditions. (In many instances, information respecting exposure chambers and gas delivery systems was limited, and often target exposure concentrations were not confirmed analytically. This raised concerns with respect to the reliability of the study findings).

In addition, many of the studies were directed more at understanding the mechanism of action of H_2S, especially at the cellular level, than at identifying health effects *per se*. In this regard, the primary focus of close to 70 percent of the studies was to examine "biochemical" or "metabolic" changes in response to H_2S exposure (*e.g.* changes in enzyme activities, changes in lipid, carbohydrate and/or protein metabolism). Although the basis for the conduct of these studies was often explained and justified by the study investigators, the toxicological significance and clinical relevance of the observed changes were not readily apparent in most instances.

The health effects information obtained from the studies was organized and summarized on a system-by-system basis, consistent with the approach used by the Ad Hoc Committee on H_2S Toxicity in 1988. Much of the information referred to effects of H_2S on the nervous system and respiratory system. This was not unexpected given the signs and symptoms commonly reported following H_2S poisoning (*i.e.*, symptoms consistent with nervous system involvement) and the portal of entry of the inhaled gas (*i.e.*, via the respiratory tract). A significant amount of information dealt with effects on the reproductive system following exposures to H_2S before, during and/or after pregnancy. Effects on "metabolic" systems, especially shifts from aerobic to anaerobic metabolism, were also commonly studied. Information on most other organ systems, including the skin, liver, kidney, GI tract, hematopoietic and immunologic systems, was found to be generally lacking. Principal findings are highlighted below.

Mortality

No deaths were recorded in any of the clinical or case-control studies reviewed.

In terms of animal testing, with the exception of a single study of moderate quality (Weedon *et al.*, 1940) no deaths were reported following short-term exposures to H_2S at concentrations up to 100 ppm. The exception involved the exposure of rats and mice to H_2S at concentrations up to 1000 ppm for 16 hours, with the discovery of deaths following exposure to 63 ppm for as little as one hour. In a second older study, conducted by Mitchell and Yant (1925), deaths were reported among rats, guinea pigs and dogs following prolonged exposures to 100 to 140 ppm; however, the reliability of the information was judged to be highly suspect. The weight-of-evidence suggests that death is very unlikely following short-term exposure to H_2S at concentrations less than 100 ppm.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

Signs and Symptoms

No subjective complaints were voiced by young, healthy male and female subjects following controlled exposures to H_2S at concentrations up to 10 ppm for 15 to 30 minutes. Exposures were via a specially-fitted mouthpiece, thereby precluding any responses related to eye irritation or the odour of H_2S. Exposures were performed under conditions of moderate to strenuous exercise, thereby exaggerating the amount of H_2S inhaled relative to resting conditions.

In a separate study, complaints of unpleasant odour, and dryness of the throat, with or without headache were recorded among individuals with mild to moderate asthma following "whole-body" exposure to 2 ppm of H_2S for 30 minutes under controlled conditions.

Signs and symptoms among test animals following single or repeated exposures at concentrations up to 100 ppm varied from "nothing" through hyperactivity and lethargy to unconsciousness depending on the species and exposure conditions. Signs were often transient and resolved quickly.

Eye

Complaints of eye irritation were recorded in only one of the clinical studies reviewed. The complaints were registered following brief exposure to 100 to 150 ppm of H_2S. The study was an older investigation, performed in 1925.

Fewer than 10 percent of the non-clinical studies reported signs of eye irritation, even at concentrations as high as 100 ppm. Most responses were mild and non-descript (*e.g.*, preening of the eyes and facial area, "washing" of the face). In no instance was the irritation confirmed through *in situ* ophthalmoscopic examination or post-mortem pathological evaluation.

The findings contradict those found by the Ad Hoc Committee on H_2S Toxicity in 1988. The Ad Hoc Committee reported that the eye *"is very sensitive to the irritant action of* H_2S*"* and *"irreversible eye tissue damage can occur at 20 ppm* H_2S *… "*. The discrepancy in findings was traced to a reliance on review articles by the Ad Hoc Committee in which unsubstantiated opinions regarding the sensitivity of the eye to H_2S were propagated.

Respiratory System

Clinical studies involving exposure of young, healthy individuals to H_2S at concentrations up to 10 ppm for 15 to 30 minutes while performing mild to strenuous exercise revealed no airway symptoms nor changes in pulmonary function. Exposure of individuals with mild to moderate asthma to 2 ppm of H_2S for 30 minutes produced mixed results, with 20 percent of the subjects (*i.e.*, 2 of 10) showing changes in pulmonary compliance consistent with bronchial obstruction. Airway symptoms were absent in all subjects.

Effects of short-term exposure to H_2S on the respiratory system noted in animal tests were confined to modest changes in the architecture of the lining of the respiratory tract among rats exposed to 80 ppm. The changes were generally transient, with recovery and/or repair of the tissues noted within a few days post-exposure. The lining of the nasal passages was most affected, consistent with the fact that rats are obligate nasal breathers.

Nervous System

Signs and symptoms consistent with nervous system involvement were generally absent from the clinical studies. Apart from complaints of headache among 3 of 10 individuals with mild to moderate asthma following exposure to 2 ppm of H_2S for 30 minutes, no other indications of nervous system involvement were reported.

With respect to the non-clinical studies, signs and symptoms suggestive of nervous system involvement varied by study, and ranged from "nothing" to agitation, hyperactivity, lethargy, drowsiness or unconsciousness. In many cases, the symptoms were transient, and disappeared during the exposure period or shortly thereafter.

A number of "biochemical" and functional changes within the brain and nervous tissues of rats, mice or guinea pigs were reported following short-term exposure to H_2S in the concentration range of interest (*i.e.*, 0 to 100 ppm). The changes included shifts in lipid, protein and RNA content, altered enzyme activities, and irregularities in EEG activity. The biological significance and clinical relevance of these changes were not readily apparent. In most instances, the findings originated from studies assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and/or reporting.

Reproductive System

No eligible studies on the effects of short-term exposure to low levels of H_2S on human reproductive performance and outcome were identified.

No reliable evidence to suggest that H_2S is a reproductive toxin or teratogen emerged from the non-clinical studies. In the most definitive study of this type, rats exposed to H_2S at concentrations up to 80 ppm before, during and after gestation showed no changes in reproductive performance indices (*e.g.*, fecundity, fertility, gestation time, number of implantations, number of resorptions, litter size). No structural changes were observed among the fetuses. Growth and development of the offspring were normal, as judged by evaluation of developmental landmarks and behavioral testing.

Several studies reported "biochemical" changes within the developing nervous tissues of neonates following *in utero* exposure to H_2S at concentrations ranging from 20 to 75 ppm. The changes, which consisted of possible shifts in neurotransmitter levels as well as alterations in protein and lipid metabolism, were of unknown biological significance.

Olfactory System

In terms of clinical studies, responses involving the olfactory system were limited to complaints of an *"unpleasant smell"* from individuals with mild to moderate asthma during exposure to 2 ppm of H_2S for 30 minutes. The smell was transient, with the subjects becoming accustomed to the odour within a few minutes after the start of exposure.

In non-clinical studies, rats exposed to H_2S at concentrations up to 100 ppm showed no structural changes in the architecture of the nasal olfactory epithelium; however, cytochrome oxidase residing within the olfactory epithelial tissues was inhibited at 80 ppm. The latter finding might account for the sensitivity of the olfactory neurons, and possibly provides an explanation for the olfactory paralysis reported to occur at higher concentrations of H_2S (*i.e.*, 150 to 200 ppm).

Metabolic Systems

Clinical studies involving the exposure of young, healthy and "aerobically fit" male and female subjects to H_2S at concentrations up to 10 ppm for up to 30 minutes demonstrated a possible shift from aerobic to anaerobic metabolism, as evidenced by increased oxygen uptake, elevated blood lactate levels and/or changes in the activities of certain enzymes involved in the tricarboxylic acid (TCA) cycle. The shift was modest, and was not evident among the female subjects. Nevertheless, it was consistent with expectations given the mechanism of action of H_2S (*i.e.*, inhibition of cytochrome oxidase). The clinical relevance of the observed changes was judged to be questionable given the modest nature of the responses and the fact that shifts to anaerobic metabolism occur regularly in response to increased tissue oxygen and energy demands.

Skin and Integumentary System, Cardiovascular System, Kidney, Liver, Gastrointestinal System, Hematopoietic System and Immunological System

Little information was found in the published literature concerning the effects of short-term exposure to H_2S at concentrations in the 0 to 100 ppm range on these systems. Any information that was available was generally non-descript and/or non-specific. No evidence emerged to suggest that these systems were especially responsive to H_2S, but this needs to be interpreted with caution in light of the limited published information that was found.

Conclusions

Conclusions reached concerning the health effects of short-term exposures to low levels of H_2S were separated to distinguish between the evidence gathered from clinical versus non-clinical studies. The conclusions are presented below.

Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations could not be firmly established.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting. The toxicological significance and clinical relevance of the responses was not readily apparent.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects remained unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms that did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al.*, 1940). In a second older study conducted by Mitchell and Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is remote.

No indications of significant effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed before, during and after gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species were not found.

It must be emphasized that no attempt was made in the present review to extrapolate the findings from the animal studies to the human condition. Specifically, no attempt was made to adjust the findings to account for differences between species in factors such as pulmonary dynamics that would act to determine the "toxic load" of H_2S received in each of the studies reviewed. Similarly, no attempt was made to extrapolate the findings from the clinical and case-control studies to the general population. Thus, caution must be exercised in the interpretation and use of the conclusions.

I. BACKGROUND

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations-Final Report.* The report contains a series of recommendations aimed at increasing awareness of sour gas and its potential impacts on public health, improving the regulatory system surrounding sour gas, and fostering better communication between all affected stakeholders on matters relating to sour gas.

Among the 87 recommendations contained in the report, several relate to the need to increase awareness of the potential health hazards of sour gas *and* to ensure that regulatory standards reflect current understanding of the health effects information on sour gas and its constituents. Two of these recommendations were central to the present work. They were:

9. *The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed, as soon as practical due to its urgency.*

59 *The EUB work with Alberta Health and Wellness, regional health authorities, and other stakeholders to develop clear requirements and evacuation criteria to address the hazard of* SO_2 *as a result of ignition.*

In response to *Recommendation 9*, Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). At the same time, AH&W agreed to accept responsibility for performing a similar review for sulphur dioxide (SO_2), in partial fulfillment of *Recommendation 59.* In keeping with the mandate of the Provincial Advisory Committee, the focus of the reviews was to be on the health effects of "acute" or short-term exposures to H_2S and SO_2. By way of direction, the Advisory Committee referred to the *"table of effects of* H_2S*"* prepared by the Ad Hoc Committee on H_2S Toxicity in 1988 (Alberta Health, 1988) as a possible starting point for the review, and urged that a revised table be developed as soon as possible.

In fulfilling its commitments, Alberta Health and Wellness elected to retain Cantox Environmental Inc. ("the consultant") of Calgary, AB to perform the reviews of the health effects information. On the recommendation of the consultant, AH&W commissioned an Expert Panel to assist in defining the scope and nature of the work and to provide technical advice for the performance of the reviews. The Expert Panel consisted of members drawn from Alberta Environment, regional health authorities, industry and other interested stakeholders. In order to ensure that the performance of the work took advantage of different perspectives and expertise surrounding the potential health hazards of sour gas.

This report focuses on the review of the health effects information currently available on H_2S. A separate report will be prepared on the health effects of SO_2. In order to maintain some degree of continuity, this report references the earlier report prepared by the Ad Hoc Committee on H_2S Toxicity on behalf of Alberta Health and Wellness (1988). A similar format has been followed to ease review and interpretation of the findings and conclusions.

The work commenced in October 2001 with the inaugural meeting of the Expert Panel. Terms of Reference were finalized late in March 2002. The review of the health effects information began in April 2002. The work was completed in July 2002 with the issuance of this final report summarizing the health effects information.

II. INTRODUCTION

At the request of Alberta Health and Wellness (AH&W), a comprehensive review of the currently available scientific literature on the health effects of hydrogen sulphide (H_2S) was undertaken. The review focused specifically on the health effects following short-term exposures to low concentrations of H_2S. To ensure full understanding of the nature and scope of the review, the following definitions were developed:

short-term, *adj.* **1.** extending over a few hours or a few days. **2.** encompassing acute and sub-acute events lasting up to 30 days.

low concentrations, *n, pl.* concentrations ranging from 0 to 100 parts-per-million (ppm) in air.

health effect, *n.* an undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.

Cantox Environmental ("the consultant") was retained by Alberta Health and Wellness to complete the review. In keeping with the need expressed by the Provincial Advisory Committee on Public Safety and Sour Gas to work with Alberta Environment, regional health authorities, industry and other stakeholders, an "Expert Panel" was commissioned to provide advice and direction regarding the performance of the work.

The members of the Panel were[5]:

Dr. Randy Angle
(Alberta Environment)

Dr. Nicholas Bayliss
(Alberta Health and Wellness)

Dr. Donald Davies ("consultant")
(Cantox Environmental Inc.)

Dr. Stephan Gabos
(Alberta Health and Wellness)

Dr. Brent Friesen
(Calgary Health Region)

Mr. Geoffrey Granville
(Shell Canada Ltd.)

Dr. Paul Hasselback
(Chinook Health Region)

Mr. Alex MacKenzie
(Alberta Health and Wellness)

Dr. Ingrid Vicas
(Calgary Health Region – Alberta Poison Centre)

The specific roles performed by the Expert Panel were:

- To provide advice and direction in relation to the overall scope and nature of the work.
- To assist in the development of the exact Terms of Reference for the work.
- To assist in identifying scientific literature pertaining to the health effects of H_2S.
- To assist in the development of criteria for gauging the technical quality of the scientific literature.
- To provide technical advice, as needed, to assist the consultant in the grading and interpretation of the literature.
- To audit the work performed by the consultant to ensure objectivity, consistency and rigour in the grading and interpretation of the scientific literature.
- To review and comment on progress reports developed by the consultant.

The Panel met on seven occasions during the course of the work, in October 2001, January 2002, February 2002, March 2002, May 2002, early June 2002 and late June 2002.

Much discussion surrounded the Terms of Reference for the work to ensure a clear understanding on the part of the consultant and Panel members as to the exact scope and nature of the review. As indicated already, it was determined that the review would focus on the health effects associated with short-term exposures to low concentrations of H_2S. The exact Terms of Reference are outlined in Chapter III.

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[5] The full titles and affiliations of the Panel members can be found in Appendix 1.

In completing the review, a comprehensive search of published scientific literature was performed in order to identify, as best possible, all available scientific papers dealing with the health effects of short-term exposure to H_2S. Eligibility criteria were developed to narrow the list of studies to those satisfying the Terms of Reference. Studies meeting the eligibility criteria were then subjected to a detailed and thorough review, including an assessment of the technical quality of the work based on consideration of experimental design, conduct and reporting. Interpretation of the biological significance and clinical relevance of the findings from each study formed part of the review. The studies covered a broad spectrum of investigations and included non-clinical studies involving exposure of test animals under controlled experimental conditions, clinical studies with volunteer human subjects exposed under controlled conditions, and case-control studies involving exposure of humans as a result of accidental releases of H_2S. In conducting the review, the consultant relied on technical expertise as well as professional judgement to arrive at conclusions. A deliberate and conscious attempt was made to ensure that the review was performed with full respect for the need for objectivity, consistency and care in the interpretation of the data.

III. TERMS OF REFERENCE

Work aimed at developing the Terms of Reference for the review began in October 2001. The final Terms of Reference were reached in March 2002. During the course of this period, the Expert Panel met on four occasions to debate the exact Terms of Reference to be followed. Agreement was reached on the following terms:

- The review was to focus on the health effects following short-term exposure. The term *"short-term"* was to include exposures of both an acute and subacute variety, to capture exposures lasting a few hours to a few days. The subacute category was further defined to include exposures extending up to 30 days.
- The review was to focus on health effects following 'low dose' exposures. The term *"low dose"* was to include concentrations of H_2S up to and including 100 ppm. Effects observed at higher concentrations were to be included at the discretion of the consultant, subject to agreement by the Expert Panel.
- The review was to focus on health effects *per se*. Although a formal definition of *"health effects"* was not adopted by the Expert Panel, the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*
- The review was to give special consideration to the potential health effects of H_2S on the developing fetus in light of the potential increased sensitivity of this life-stage to the harmful effects of chemicals, and the concerns often expressed during pregnancy over chemical exposures.
- The review was to be limited to peer-reviewed scientific publications. Preference was to be given to English-language journals, with specific foreign-language papers to be reviewed at the discretion of the Expert Panel.
- The review was to include *all* currently and readily available journal articles, with a strict need to avoid possible journal and/or sponsor bias.
- The review was to focus on scientific studies involving exposures to H_2S via inhalation to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (*e.g.*, oral, dermal, injection) were to be excluded from review.
- The review was to focus on full-length primary scientific papers describing original work rather than on review articles and abstracts.
- The review was to include consideration of clinical investigations involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory, and "population" studies involving exposures following routine or accidental releases of H_2S into the environment.
- The review was to include a critical assessment of the technical quality of each scientific paper based on consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

Further discussion surrounded the need for each scientific paper to be subjected to independent review by two members of the consultant's team to ensure objectivity, consistency and fairness in judging the technical quality of the scientific studies, and consistency and rigour in the interpretation of findings and conclusions.

Additional discussion centered on the need for independent audit of the consultant's work, with agreement reached that the members of the Expert Panel would perform separate reviews of selected scientific papers to ensure that the consultant's findings fairly represented the strengths and weaknesses of each reviewed document.

Much discussion was devoted to distinguishing between *"science"* and *"policy"* as they related to the performance of the work, with the understanding that linkages invariably exist between the two elements. It was agreed that the review of the health effects information would be strictly a scientific exercise in which the quality of the information would be assessed against a set of technical criteria and the scientific meaning of the study findings would be presented, without consideration of the public health implications. Interpretation of the biological significance and clinical relevance of the findings would be allowed, but not interpretation of the significance of the findings for the purposes of setting policy for the protection of public health.

The question of the need to assess the possible health implications associated with the odour of H_2S was discussed at length. It was acknowledged that concern over the odour of H_2S represents a valid and important issue given the reported physiological and/or psychological responses that can be elicited through the sense of smell, the very low odour threshold for H_2S, and the number of odour complaints that historically have been registered in Alberta in relation to sour gas operations. Much discussion centered on whether or not the detection of an unpleasant odour in and of itself constitutes a health effect *per se*. Differing opinions were expressed in this regard. Some Panel members held that an odour, be it pleasant or unpleasant, simply represents a response to a stimulus, and as such, even an unpleasant odour does not qualify as an undesirable or harmful effect. Other Panel members argued that, although the primary response to an odour may not qualify as an adverse effect, the secondary responses that the odour might trigger could prove to be undesirable or harmful. Despite the differing opinions, it was agreed that the review of the health effects information would not specifically address the potential physiological or psychological responses that might be elicited by unpleasant odors, such as the characteristic "rotten egg" smell of H_2S. However, at the same time, it was determined that the review should carefully document studies for which odour complaints were recorded, including a description of any signs and symptoms that emerged which may have been related to the unpleasant smell of H_2S.

Some discussion centered over the need to clarify issues surrounding the effects of exposure to low levels of H_2S on "hypersusceptible" individuals[6], based on reports suggesting that certain individuals may be especially responsive to H_2S owing to pre-existing medical conditions. Agreement was reached that emphasis would be directed at assessing the effects of exposure to H_2S on normal populations, but information which emerged during the review which related to the issue of hypersusceptibility, as defined by the Illinois Institute for Environmental Quality (IIEQ, 1974), also would be captured. The need to consider such information respected the fact that a significant portion of the general population may have pre-existing health problems, such as cardiovascular or respiratory disease, that may increase susceptibility to H_2S.

In addition, some discussion focused on the need to consider reports of health effects following intermittent "peak" exposures to H_2S occurring against a chronic low-level "background" of exposure, as might occur for individuals living downwind of industrial (*e.g.*, pulp mills) or agricultural (*e.g.*, feedlots) sources. Agreement was reached that this type of exposure scenario did not satisfy the definition of "short-term" adopted for the purposes of the present review.

Finally, some discussion revolved around the need to include the effects of short-term exposures to low concentrations of H_2S on livestock as part of the review. It was agreed that the review would focus only on the potential effects on humans since work relating to the effects on livestock would be addressed through a separate initiative (*i.e.*, the Western Inter-provincial Scientific Studies Association – WISSA- initiative). It was understood that the potential effects on humans would be assessed through consideration of clinical, non-clinical, and case-control studies involving short-term exposures to low levels of H_2S. With respect to the non-clinical studies, emphasis was necessarily to be given to tests performed with conventional laboratory animal species, such as rats, mice and guinea pigs.

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[6] The term "hypersusceptible" was used in the context of the description provided by the Ad Hoc Committee on H_2S Toxicity (Alberta health, 1988) and referred to individuals who might be at increased risk due to pre-existing medical conditions that could be aggravated by exposure to H_2S. The term was not used to describe individuals who may be intrinsically more sensitive to chemicals in general and might respond to lower concentrations of H_2S than the general population.

In completing the work, some reliance on professional judgement was needed in order to grade the technical quality of the studies and to interpret the biological significance and clinical relevance of the findings and conclusions. The consultant was cautioned by the Expert Panel of the absolute need for objectivity, the need for independent appraisal, and the need to limit opinion.

IV. METHODS

The work proceeded in stages beginning with a comprehensive search of the published scientific literature to identify studies in which the health effects of short-term exposures to low concentrations of H_2S were investigated. The scientific papers describing the studies were then retrieved and catalogued. Each study was subsequently subjected to a detailed review, which first encompassed an evaluation of the technical quality of the investigation based on consideration of experimental design, conduct and reporting. A set of "quality" criteria was developed for each of the various study types (*i.e.*, clinical, non-clinical, and case-control) to permit objective and consistent judgement of technical strengths and weaknesses. The criteria were based on the recommendations of leading scientific authorities for the proper design, execution and reporting of health effects studies. A grading system was established and, on the basis of the initial evaluation of technical quality, each study was assigned a confidence index ranking (*e.g.*, low, moderate or high) that reflected the level of confidence that could be assigned to the findings and conclusions. A detailed assessment of the observations recorded from each study was then performed, with emphasis placed on those studies which achieved a moderate or high grade. The information from each study was summarized and captured in a "document review form" developed specifically for this work (see Appendices 5 and 6). The form contained details concerning study design features, observations, authors' conclusions, and strengths and weaknesses vis-à-vis technical quality. Interpretation of the biological significance and clinical relevance of the study findings also formed part of the review. A description of the various stages comprising the work follows.

A. Search Strategy

As indicated earlier, the first stage of work involved a comprehensive search of the published scientific literature to identify, as best possible, all studies dealing with the health effects associated with short-term exposure to low concentrations of H_2S. Three search methods were used:

- On-line electronic databases
- Citation sourcing
- Personal communication

A brief description of each method is found below.

On-line electronic databases

A series of search terms was developed, matching the overall Terms of Reference for the work and satisfying a set of eligibility criteria that were developed for studies to be included in the review. The eligibility criteria are shown below. Search terms were broadened at the discretion of the consultant to ensure that the search was complete. Possible bias in the selection of terms was avoided by the involvement of 3 individuals in the final choice of terms. A full listing of the search terms can be found in Appendix 2.

Eligibility Criteria[1]	
Study classification	Primary research (*i.e.*, papers describing original work)
Study type	All (*i.e.*, clinical, non-clinical, case-control, *etc.*)
Exposure duration[2]	0 to 30 days (*i.e.*, acute and sub-acute)
Exposure concentration[2]	0 to 100 ppm of H_2S
Exposure route	Inhalation
Publication type	Full-length peer-reviewed publications
Other	English-language
Additions	Reproductive and developmental toxicity studies

[1] The eligibility criteria for the selection of studies to be reviewed reflected the Terms of Reference for the work, and were determined collectively by the members of the Expert Panel.

[2] Studies for which exposure duration and/or exposure concentration(s) were not specified were deemed to be ineligible and were excluded from review.

Searching was performed using the DIALOG Information Retrieval Service. The following electronic databases were accessed:

- BIOSIS PREVIEWS (1993 to 2002)
- ENVIRONMENTAL BIBLIOGRAPHY (1966 to 2002)
- ENVIROLINE (1975 to 2002)
- LIFE SCIENCES COLLECTION (1982 to 2002)
- MEDLINE (1966 to 2002)
- POLLUTION ABSTRACTS (1970 to 2002)
- TOXFILE (1966 to 2002)

Study titles and/or abstracts were downloaded and then reviewed against the Terms of Reference for the work. Studies bearing titles or abstracts that clearly met the eligibility criteria were retrieved and collated. For cases in which the match between the study title and Terms of Reference was equivocal or not readily obvious, the study was ordered and then accepted or rejected following a preliminary review.

Citation sourcing

Readily available review articles on the health effects of H_2S were retrieved and the bibliographies were manually searched for citations matching the eligibility criteria for the review. The search was completed, in part, to confirm the adequacy and completeness of the electronic search. Very few new studies were identified. A complete listing of the bibliographies that were reviewed is provided in Appendix 3.

Personal communication

A full listing of the studies meeting the eligibility criteria was provided to each member of the Expert Panel, with a request to review the list for completeness and accuracy based on his/her personal knowledge and/or records of studies on the health effects of H_2S.

Based on the three search methods, a total of 45 studies satisfying the eligibility criteria were identified, retrieved and subjected to detailed review.

B. H_2S Database

As indicated earlier, the Terms of Reference for the work evolved over a six-month period, during which time the eligibility criteria for the studies to be included in the review shifted as new information became available. In order to accommodate the shifts, and out of respect for the large body of information that exists on the health effects of H_2S, a computerized bibliographic database was developed to allow for the orderly sorting and classification of the published studies identified through the search strategy. A bibliographic form was created which captured pertinent information respecting study identification, source, type, design features *etc.* (see Appendix 4). A separate form was completed for each study and stored in the database. The database was designed to permit the entire complement of studies to be searched against the eligibility criteria. As a result, shifts in the criteria as the Terms of Reference for the review evolved were easily accommodated. More than 600 studies were entered into the database. A search against the final set of eligibility criteria pinpointed the 45 studies that were subjected to detailed review. A CD version of the database can be made available upon request. Requests should be directed to Alberta Health and Wellness.

C. Quality Criteria

Each of the 45 studies meeting the final set of eligibility criteria was subjected to a detailed review and evaluation. As part of the review, the overall technical quality of the study was assessed based on consideration of experimental design, conduct and reporting. This step of the review was deemed to be important since it had a direct bearing on the level of confidence that could be assigned to the study findings and conclusions. A set of quality criteria was developed for each study type (*i.e.*, non-clinical, clinical, case-control) on the basis of the recommendations of leading scientific and regulatory authorities for the proper design, execution and reporting of health effects studies (Clarke and Oxman, 2001; OECD,1993; USEPA, 1998). The recommendations were captured in a series of "check-lists" organized in a Q&A format, against which the studies were compared. Full copies of the check-lists can be found in Appendix 5. A sample of the Q&A format is shown opposite.

Judgement of the technical quality of the studies against a pre-defined set of quality criteria that reflected the opinions of leading experts helped to ensure that the reviews proceeded in an objective, consistent and fair manner. A grade was assigned to each study depending on the outcome of the review. Grading necessarily involved weighing the strengths and weaknesses of the study, using the appropriate check-list for guidance. Grades were expressed as a "Confidence Index Ranking" to indicate the overall level of confidence that could be given to the findings and conclusions. The rankings included:

- *High* – Signifying that the study meets or exceeds the recommended guidelines, with no serious weaknesses in experimental design, conduct or reporting. Procedures are well-described and results are properly disclosed to permit meaningful interpretation. Study validity is obvious. Confidence in the findings and conclusions is high.
- *Moderate* – Signifying that the study generally subscribes to the recommended guidelines, but minor deficiencies in design, conduct or reporting detract from the interpretation of the results. Study validity is evident, but not obvious. Careful attention to detail in describing procedures and presenting results may be lacking. Confidence in the findings and conclusions is somewhat restrained, but not weak.
- *Low* – Signifying that the study fails to meet the recommended guidelines and serious weaknesses in design, conduct and reporting are evident. Significant departures from the recommended guidelines may be present. Sufficient detail is lacking to permit meaningful interpretation of results. Study validity is questionable. Confidence in the findings and conclusions is low.

A great deal of care was taken in the assignment of confidence index rankings out of respect for the need to ensure objectivity, consistency and fairness. The ranking relied on the consultant's technical expertise, familiarity with the recommended guidelines, and professional judgement.

Sample Check-list

Non-clinical study – Exposure conditions:

☐ Was a full description (manufacturer, purity, lot no.) of the reagents (including H_2S) used in the trial provided?

☐ Was there a gradient of exposure levels?

Guideline: Three exposure levels in addition to control.

☐ Were the chosen exposure levels appropriate to investigate the primary objective?

☐ Were previous preliminary trials conducted to identify the range of appropriate exposure levels?

☐ Were both nominal and actual exposure concentrations recorded?

☐ Was the duration of exposure appropriate to investigate the primary objective?

☐ Was the duration of exposure precisely defined?

☐ If exposure chambers were employed, were animals exposed during the equilibration period?

Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.

☐ Was the mode of administration appropriate?

☐ Was the frequency of administration appropriate to investigate the primary objective?

☐ Was evidence provided to justify the dosing regime?

☐ Were exposure concentrations, chamber airflow, temperature and humidity monitored on a regular basis?

D. Review Process

Each study was subjected to independent review by at least two members of the consultant's team. The primary reviewers were:

Dr. Donald B. Davies, Ph.D., DABT
Vice-President, Scientific Programs

Ms. Susan E. Haggarty, B.Sc.
Environmental Risk Analyst

In the event of disagreement between the two reviewers over the interpretation of the study findings or the assignment of the confidence index ranking, a third reviewer was engaged and any differences were resolved through discussion among all three individuals. Disagreement was rare. In virtually all cases, the confidence index ranking was obvious.

The review began with the assessment of the technical quality of the studies, and then proceeded to the interpretation of the study findings and conclusions. Emphasis was given to those studies which achieved a moderate or high confidence index ranking. On the advice of the Expert Panel, studies that received a "low" ranking were still considered in the overall interpretation of the health effects information but these studies received less emphasis. The detailed review of the studies commenced in early April 2002, and was completed by July 2002.

V. SUMMARY OF HEALTH EFFECTS

A. Overview

The Terms of Reference for the work were to identify and assess the health effects associated with short-term exposure to H_2S at concentrations up to 100 ppm. As indicated earlier, "short-term" exposure referred to exposures of an acute or subacute nature, extending from a few hours up to 30 days. Special consideration also was given to the potential effects of H_2S on the developing fetus, in which case, exposures necessarily extended over several weeks to capture the gestation and post-natal periods. Although emphasis was directed at exposure concentrations in the 0 to 100 ppm range, observations from studies involving exposures to graded concentrations within *and* above the range were recorded in order to provide a more comprehensive survey of potential health effects.

Each study satisfying the eligibility criteria described earlier (Chapter IV) was subjected to a detailed review. The review encompassed the following:

- Determination of study objective(s);
- Review of principal findings and conclusions;
- Review of strengths/weaknesses in study design, conduct and reporting;
- Ranking or "scoring" of the study on the basis of the level of confidence that could be assigned to the findings and conclusions; and
- Assessment of the toxicological significance and clinical relevance of the study findings.

The ranking of the studies relied on a pre-determined set of "quality criteria" (Chapter IV) that were based largely on the recommendations of several leading scientific authorities for the proper design, execution and reporting of clinical and non-clinical studies (*i.e.,* Clarke and Oxman, 2001; OECD, 1993; USEPA, 1998). Ranking was completed with full respect for the need for consistency, objectivity and fairness, and followed a weight-of-evidence approach in which study strengths and weaknesses were carefully measured. Each study was subjected to two independent reviews. A written summary capturing the highlights of each study, complete with a "Confidence Index Ranking", was prepared. The full set of summaries can be found in Appendix 6.

Readers are strongly encouraged to review the study summaries found in Appendix 6 to obtain detailed information with respect to the findings and conclusions from each study, as well as details concerning study design, conduct and reporting.

A compilation and summary of the reported health effects associated with short-term, "low dose" exposures to H_2S is presented below. The effects are arranged on a system-by-system basis and are distinguished by study type (*i.e.,* clinical, non-clinical, case-control). Emphasis was placed on those studies which achieved a "Confidence Index Ranking" of "high" or "moderate", signifying that the study satisfied most quality criteria and showed no serious deficiencies in design, conduct and reporting. Less emphasis was given to studies assigned a "low" rating since little confidence could be placed in the findings and conclusions. A listing of "positive" findings from the various studies (*i.e.,* evidence of health effects), together with the corresponding exposure concentrations is attached (see Figure 1). A companion listing of "negative" findings (*i.e.,* absence of health effects) is captured in Figure 2. Both figures have been included to permit more meaningful interpretation of the findings from a weight-of-evidence perspective. Two separate figures (Figures 3 and 4) listing the principal findings relating to reproductive and developmental toxicity also are included. The level of confidence assigned to each set of findings is indicated. It must be emphasized that the information contained in each of these figures should *not* be considered in isolation. Rather each entry in the figures should be reviewed in combination with the corresponding written summary of the study, as well as the information that follows to ensure that the biological significance and clinical relevance of the finding(s) are understood. Otherwise, the entries could be easily misconstrued.

It also must be emphasized that, although the information respecting the health effects associated with short-term exposure to H_2S is distinguished by study type (*i.e.*, clinical, non-clinical and case-control), no attempt has been made to extrapolate the findings from the animal studies to the human condition. Such an exercise would require careful consideration of the "toxic load" received by each test specie under the various exposure conditions studied. "Toxic load" is the mass (or flux) of H_2S delivered to the target site(s), which is a function of concentration and time, and differs across exposed species for physiological reasons. The determination of "toxic load" would necessarily depend upon:

- Exposure concentration(s).
- Duration of exposure.
- Specie-specific pulmonary dynamics (*e.g.*, breathing patterns, breathing rates).
- Differences in pharmacokinetic profiles of H_2S between species (*e.g.*, absorption, distribution, metabolism and excretion patterns).
- Architectural variations in the respiratory tract of different species that could affect the deposition pattern and ultimate disposition of any inhaled H_2S.
- Relative life-span of each test specie, particularly for studies involving repeated exposures extending over several days.
- Other specie variations.

Thus, care should be taken in the interpretation of the health effects information, particularly with respect to extrapolation of the findings to the general human population.

B. General Comments

The review of health effects information has been organized on a system-by-system basis, consistent with the approach used earlier (Alberta Health, 1988). For ease of review, studies were segregated by study type (*i.e.*, non-clinical, clinical and case-control). Similarly, given the extensive amount of information that was available for some systems, the information is arranged according to the nature of the effects (*i.e.*, signs and symptoms, functional changes, structural changes, biochemical changes). A total of 45 studies met the eligibility criteria and were subjected to detailed review and assessment. Some general comments concerning the study "sample" follow:

- Non-clinical investigations involving controlled exposure of test animals comprised the greatest number of studies (75%). Most of the remaining studies involved clinical trials with volunteer human subjects exposed to H_2S under controlled conditions (15%). Only four "case-control" studies were identified and reviewed. The preponderance of non-clinical tests presents the challenge of extrapolating the findings to the human condition, and heightens the difficulty of assessing the public health implications of the results.
- Very few studies achieved a "high" confidence index ranking (15%), in which the experimental design, conduct and reporting were well-described and in good agreement with the recommendations found in testing guidelines. Approximately 40% of the studies achieved a "moderate" rating, signifying some weaknesses that undermined confidence in the findings and conclusions. The remaining studies (45%) were found to be deficient, with findings and conclusions that could not be supported owing to serious limitations in experimental design, conduct and/or reporting. These studies were assigned a "low" rating. It should be noted that the preponderance of "low" scores was not the product of overly strict quality criteria, but rather reflected a general lack of attention to detail in the studies and a failure to subscribe to standard operating procedures and conventional testing protocols. Common deficiencies which hindered the interpretation of study findings are shown on the following page.

Common Deficiencies of "Low" Rated Studies

Clinical:

- Limited number of subjects
- Lack of reliable exposure estimates

Non-clinical:

- Failure to follow conventional testing protocols
- Use of too few test animals
- Use of a single sex only
- Inadequate details concerning animal housing, husbandry, and handling
- Use of a single exposure concentration only
- Inadequate description of exposure conditions
- Failure to confirm target exposure concentration(s)
- Failure to include conventional measures of toxicity

Case-control:

- Limited number of test subjects
- Unreliable exposure estimates
- Possible selection bias in subject recruitment
- Excessive lag time between exposure and testing
- Reliance on self-administered questionnaires
- Failure to adequately control for obvious confounding variables (*i.e.*, exposure to other chemicals)
- Reporting inconsistencies

- Only a single study evidently was performed under Good Laboratory Practice (GLP) conditions, a set of operating principles first introduced in the early 1980's as a means to enhance the reliability and reproducibility of experimental results from non-clinical toxicity studies (USEPA, 1989).The lack of attention to GLP in all of the remaining studies casts some doubt on the reliability of the findings.

- Of the non-clinical studies reviewed, very few (20%) followed a conventional study design, such as those recommended by the Organization for Economic Cooperation and Development (OECD) or the United States Environmental Protection Agency (USEPA) (OECD, 1993; USEPA, 1998). As a result, standard measures of toxicity (*e.g.*, body weights, signs and symptoms, gross pathology) were routinely omitted. This hindered interpretation of the findings as well as the comparison of results across different studies. Moreover, it limited interpretation of the biological significance of certain findings (*e.g.*, changes in biochemical indices) since symptomatic and/or pathological correlates of the changes could not be determined.

- Approximately 50% of the studies were not intended to examine the health effects *per se* of H_2S. Rather, the primary objective was often to advance understanding of the mechanism of action of H_2S through the study of various physiologic, metabolic and/or biochemical indices. In many cases, the toxicological significance and clinical relevance of the observed changes in these indices was not readily apparent. In most instances, routine measures of toxicity were not included in the overall experimental design, thereby precluding assessment of the significance or relevance of the changes through consideration of symptomatic or pathological correlates.

- A sizeable number of the studies (45%) used only a single exposure concentration of H_2S. Accordingly, the dose-responsiveness of any findings could not be assessed, thereby hindering determination as to whether or not the effects were treatment related.

- Approximately 40% of the studies involved testing using a single sex only, with males predominating. In no case was justification for the use of the one sex only provided. This deficiency extended to both the clinical and non-clinical trials. A further 30% of the studies failed to specify which sex was tested (*i.e.*, male, female or both). Both types of deficiency hindered interpretation of the significance and relevance of the study findings, and added to the difficulty of extrapolating the results to the general population.

- The study 'sample' showed significant differences in the amount of information available on the effects of short-term exposures to H_2S across different organ systems. As might be expected given its mechanism of action and the portal of entry of inhaled H_2S, a number of studies were identified in which effects on the nervous system and respiratory system were examined. However, effects on many of the remaining systems were found to have received little attention. Very few, if any, studies were available describing the effects of short-term exposures to H_2S on the liver, kidney, gastrointestinal tract, skin, hematopoietic, and immunological systems. Moreover, the majority of these studies received a "low" confidence index ranking, thereby precluding meaningful assessment of the toxicological significance and/or clinical relevance of the findings. Of the remaining studies, most provided only non-specific information that was of limited use in assessing system-specific responses.

The overall impression of the study "sample" was that weaknesses exist in the information currently available on the health effects associated with short-term, "low-dose" exposure to H_2S. In the majority of studies reviewed, conventional testing protocols were ignored and basic design elements were missing (*e.g.*, testing of graded exposure concentrations in both sexes, measurement of routine toxicity indices). Moreover, much of the information is of unknown or questionable toxicological significance as a result of an emphasis on monitoring biochemical and/or metabolic indices (*e.g.*, disturbances in enzyme activities, lipid or protein content of tissues, neurotransmitters levels, ECG or EEG tracings, serum mineral content) instead of standard toxicological endpoints. The clinical relevance of many of the findings also is difficult to assess. In many cases, the study conclusions were judged to be tenuous.

A summary of the health effects information follows. Emphasis is given to studies which were assigned a confidence index ranking of "high" or "moderate". Reference is made to studies which achieved a "low" rating only for completeness. For ease of interpretation, the 'Confidence Index Ranking' for each study is indicated in the text according to Study ID number using the following color and symbol scheme:

Confidence Index Ranking	
High or high-to-moderate	▲
Moderate-to-high, moderate or moderate-to-low	◆
Low or low-to-moderate	●

Summary remarks are included at the end of each section to assist in the interpretation of the various study findings.

C. Mortality

Clinical studies

All clinical studies involving human subjects were free of deaths, as would be expected given the low exposure concentrations used ($\leq$ 30 ppm) and the administrative controls in place.

Non-clinical studies

Mortality was rarely observed following inhalation exposure to H_2S at concentrations up to 100 ppm. However, Weedon *et al.* (1940) did report deaths among rats and mice following "whole body" exposure to 63 ppm of H_2S for up to 16 hours under controlled conditions. The mice were more markedly affected than the rats, with 4 of 4 mice dying within as little as one hour after onset of exposure, and 1 of 8 rats dying within 16 hours. All remaining rats survived. Gross findings recorded at necropsy for the animals dying on test revealed hemorrhagic infiltration of the lungs and congestion of the brain, liver and/or kidneys consistent with generalized systemic toxicity. No deaths occurred in rats and mice exposed to 16 ppm of H_2S in the same study. At 250 ppm, 3 of 8 rats and 4 of 4 mice were found dead within several hours. At 1000 ppm, all animals died within one hour of exposure. The study was assigned a confidence index ranking of "moderate", with no serious weaknesses noted in experimental design, conduct and reporting. However, the study was "dated", and the exposure chamber, gas delivery system, and monitoring equipment were very basic compared to present-day standards (Study ID ◆ 316).

In a second older study, Mitchell and Yant (1925) reported deaths among rats exposed "continuously" to 100 to 140 ppm of H_2S for 18 to 48 hours. The incidence of deaths could not be determined. No deaths were recorded following continuous exposure of rats to 35 to 65 ppm of H_2S for up to 100 hours. In the same study, 1 of 2 guinea pigs and 1 of 2 dogs were found dead following continuous exposure to 103 ppm of H_2S for 18 to 48 hours and 8 to 16 hours, respectively.

All guinea pigs survived exposures to 35 to 65 ppm of H_2S. At higher concentrations (*i.e.*, >100 ppm), a higher incidence of mortality and/or shorter time to death was recorded for all three species tested. Canary birds were found to be especially sensitive to H_2S, with deaths recorded within 8 to 18 hours following continuous exposure to 35 to 65 ppm. The study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting. Accordingly, caution must be exercised in the interpretation of the significance of the findings (Study ID ● 444).

No other studies reported deaths in the 0 to 100 ppm range, including studies using mice (Elovaara *et al.*, 1978 – Study ID ● 155; Savolainen *et al.*, 1980 – Study ID ● 280). Deaths were reported at exposure concentrations of 300 to 500 ppm and greater in several animal species including rats (Lopez *et al.*, 1986 – Study ID ◆ 466; Khan *et al.*, 1990 – Study ID ◆ 210; Struve *et al.*, 2001 – Study ID ◆ 296), pigs and rabbits (O'Donoghue, 1961 – Study ID ● 255).

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

Case-control studies
No deaths were reported in any of the case-control studies reviewed.

Summary:
Based on the weight-of-evidence, the likelihood of mortality occurring in response to short-term exposure to H_2S at concentrations up to 100 ppm is remote.

Clinical studies:
No deaths were reported in any of the clinical studies reviewed.

Non-clinical studies:
Deaths were rare following short-term exposures to low levels of H_2S. Deaths of rats and mice were recorded by Weedon et al. (1940) following exposure to 63 ppm of H_2S from as little as one hour to up to 16 hours. Deaths of rats, guinea pigs and dogs were reported by Mitchell and Yant (1925) following continuous exposures to 100 to 140 ppm of H_2S for at least 8 hours. No other deaths occurred in any of the remaining studies reviewed at concentrations up to 100 ppm, even when exposures extended over several hours per day for several days. The significance of these findings is discussed in Chapter VI (Other Considerations).

Case-control studies:
No deaths were reported in any of the case-control studies reviewed.

D. Signs and Symptoms

Although the routine observation of test subjects and/or test animals is a standard requirement of most clinical and non-clinical testing guidelines, a significant number of the studies reviewed contained no reference or description of signs and symptoms of toxicity either during or following exposure. As a result, information on signs and symptoms following short-term exposure to low levels of H_2S is somewhat limited.

Clinical studies
In most clinical trials involving exposure of human subjects to H_2S under controlled conditions at concentrations in the 0 to 10 ppm range, no complaints or symptoms of discomfort were reported.

In a series of studies aimed at elucidating the effects of H_2S on various pulmonary, metabolic and biochemical markers of aerobic versus anaerobic metabolism, Bhambhani *et al.* (1991; 1994; 1996a; 1996b; 1997) reported no complaints of discomfort, headache, nausea, sore throat or undue fatigue among male and female subjects during or following exposure to H_2S at concentrations up to 10 ppm for up to 30 minutes. Exposures were via a specially-fitted mouthpiece. The subjects performed moderate to strenuous exercise throughout the exposure period. All subjects were in good health and aerobically fit (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121, Study ID ▲ 122).

Similarly, Kangas and Savolainen (1987) reported no symptoms among test subjects following "whole-body" exposure to H_2S at concentrations up to 30 ppm for 30 to 45 minutes under controlled conditions (Study ID ● 207). However, confidence in the study was rated "low" due to serious weaknesses in reporting.

In contrast, Jappinen *et al.* (1990) reported than asthmatic subjects complained of an unpleasant odour, dryness of the nose and throat and/or headache during and after "whole body" exposure to 2 ppm of H_2S for 30 minutes. The former symptoms were generally transient and appeared only at the start of the exposure. None of the subjects suffered from severe asthma (Study ID ◆ 202).

In a study to investigate possible airway inflammation in response to exposure to swine dust, Larsson *et al.* (1994) monitored the general health status of healthy volunteers after working in a swine containment building for up to 5 hours. Measurements taken within the building revealed the presence of low concentrations of H_2S (<0.05 ppm). Subjects complained of headaches, malaise, nausea and/or drowsiness. However, the symptoms were not ascribed to H_2S given the low concentrations involved (Study ID ● 662).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S complained of eye irritation and respiratory discomfort within 2 to 15 minutes of exposure. The symptoms progressed to pain in the eyes, throat irritation, coughing, and excessive salivation with continued exposure for up to 4 hours. Loss of the sense of smell also was recorded. The authors described the tests as *"preliminary"*. The study was assigned a "low" confidence index ranking (Study ID ● 444).

It is noteworthy that any influence of odour on symptom reporting was effectively eliminated in the series of investigations by Bhambhani *et al.* through breathing exclusively via the mouthpiece.

Non-clinical studies

Signs of toxicity recorded following controlled exposures of test animals to H_2S at concentrations ranging from 0 to 100 ppm varied from no obvious symptoms, through initial hyperactivity, to loss of consciousness. For instance, Khan *et al.* (1990) reported no "marked" symptoms of toxicity among male rats exposed to 10, 50 or 200 ppm of H_2S for four hours. Regrettably, the term "marked" was not defined (Study ID ◆ 210). Similarly, Kosmider *et al.* (1967) found no observable evidence of toxicity among rabbits of both sexes exposed to 71 ppm of H_2S repeatedly for 30 minutes each day for five consecutive days (Study ID ◆ 223). Lopez *et al.* (1987, 1988) reported no signs or symptoms among male rats following a single exposure to 10 or 200 ppm H_2S for four hours (Study ID ◆ 231 and Study ID ◆ 233).

In contrast, several studies revealed systematic responses, typically with involvement of the respiratory and central nervous systems. Common signs included initial restlessness or agitation, changing to drowsiness, lethargy and fatigue, with or without laboured breathing and evidence of mild to moderate respiratory distress. The affected species included mice (Elovaara *et al.*, 1978 – Study ID ● 155; Savolainen *et al.*, 1980 – Study ID ● 280; Weedon *et al.*, 1940 – Study ID ◆ 316), rats (Lopez *et al.*, 1986 – Study ID ◆ 466; Weedon *et al.*, 1940- Study ID ◆ 316), rabbits (Kosmider *et al.*, 1971 – Study ID ● 595), guinea pigs (Haider *et al.*, 1980 – Study ID ● 179; Mitchell and Yant, 1925 – Study ID ● 444) and dogs (Mitchell and Yant, 1925 - Study ID ● 444). Exposure concentrations ranged from 20 to 100 ppm, delivered on an acute (*i.e.*, up to 16 hours) or repeated subacute (*i.e.*, once daily for up to 14 days) basis. The most marked signs of intoxication were reported by Kosmider *et al.* (1967) in a study involving exposure of rabbits to 71 ppm of H_2S for 1.5 hours. The animals reportedly lost consciousness (Study ID ● 223). The confidence index ranking assigned to this study was "low" in light of serious limitations in experimental design, conduct and reporting.

Signs and symptoms monitored as part of certain non-clinical studies included body weight, body weight gain, and feed consumption. Changes in these parameters can represent direct toxicity, or alternatively, they may simply reflect non-specific stress, whereupon the animal may refuse feed, thereby slowing growth. In a few instances, body weight losses or reduced body weight gains were reported following short-term exposure to

H_2S. Specifically, Lopez *et al.* (1986) reported an initial modest weight loss (*i.e.*, < 5%) among male rats following exposure to 40 ppm of H_2S for six hours. Growth was restored within 18 hours post-exposure (Study ID ◆ 466). Curtis *et al.* (1975) observed modest to mild reductions in body weight gain (*i.e.* 5-10 %) among pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416). As part of a reproductive and developmental toxicity study, Dorman *et al.* (2000) reported reduced feed intake, accompanied by a modest reduction in body weight gain (*i.e.*, 5-6%) among male and female rats during the first week of exposure to 80 ppm of H_2S for six hours per day as part of a two-week pre-mating period. Normal feed intake resumed by the second week of the study (Study ID ▲ 149).

Case-control studies

Case-control studies consistently revealed a number of subjective complaints allegedly caused by exposure to H_2S among the exposed cohorts. Symptoms were of both an immediate and latent variety.

Kilburn (1997) reported that subjects exposed for "hours" to H_2S at concentrations ranging from 1 to 50 ppm complained of a variety of symptoms at the time of exposure. The symptoms included loss of strength, headache, excessive fatigue, chest pain and awareness of an unpleasant odour. Latent symptoms reported by the same subjects two to six years after the exposure varied from eye, respiratory and gastrointestinal disorders (*e.g.*, eye irritation, throat irritation, dry cough, shortness of breath, indigestion, nausea, loss of appetite) through sleep disturbances and altered states of mood, to memory loss and loss of concentration (Study ID ● 216).

In a subsequent study, Kilburn (1999) reported that subjects exposed to 1 to 40 ppm of H_2S for up to one week as a result of emissions associated with a refinery explosion and fire complained of altered moods when questioned more than three years after the incident (Study ID ● 217).

As part of an inquiry into the events surrounding the "blow out" of a sour gas well near Cynthia, Alberta (*i.e.*, the Lodgepole blow-out), witnesses from the area recalled experiencing a variety of symptoms, including headache, eye irritation, nausea, loss of appetite, and diarrhea, together with a host of upper and lower respiratory tract symptoms, such as sore throat, nasal irritation, nosebleeds and shortness of breath, which they alleged were linked to H_2S exposure (Study ID ● 327).

In each of these three case studies, a "low" confidence index ranking was assigned owing to serious deficiencies in experimental design, conduct and reporting. The most notable deficiencies included lack of reliable exposure estimates, excessive lag time between exposure and the evaluation of subjects, inconsistent and possibly biased reporting of findings, possible bias in the selection of subjects, and failure to control for obvious confounding variables (*i.e.*, exposures to other chemicals).

As part of a retrospective analysis completed by Alberta Social Services and Community Health (1983) of the nature and frequency of complaints received from residents living both near and distant from the site of the "Lodgepole" sour gas well blow-out, a number of signs and symptoms were identified that appeared to correlate with concentrations of H_2S measured at several locations within the surrounding area. A total of 31 different health-related symptoms were recorded, the most common of which were "feeling sick", headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, vomiting, stomach cramps and skin allergies. Complaints of an unpleasant odour were very frequent. Peak concentrations of H_2S measured in the area ranged from 0.3 to 5.5 ppm, with mean daily average concentrations ranging from 0.01 to 0.4 ppm. Exposures extended over 2 two-week periods, during which H_2S was released directly into the atmosphere. The study was assigned a "low-to-moderate" confidence index ranking owing to several weaknesses in design, conduct and reporting. Weaknesses included lack of reliable exposure estimates, possible subject bias, possible information bias, and exclusive reliance on self-reported symptoms (Study ID ● 424).

Summary:
It is regrettable that for most of the studies

reviewed either routine observation of the subjects or test animals was not performed or the results were not reported. Based on the findings from the remaining studies, with emphasis necessarily given to the observations from those investigations which achieved a "moderate" or "high" confidence index ranking, it would appear that adverse signs and symptoms are unlikely to occur among healthy young adults as a result of short-term exposure to H_2S at concentrations up to 10 ppm.

Clinical studies:
The series of studies by Bhambhani et al. revealed that short-term exposure to concentrations up to 10 ppm can be tolerated without complaint by young, healthy and "fit" individuals, even when inhaling the gas directly while performing moderate to strenuous exercise. The lack of symptoms among the subjects was especially revealing since they were aware in advance of the clinical signs that might be experienced. However, possible symptoms related to the irritant properties of the gas cannot be dismissed since the subjects breathed through a mouthpiece. Some asthmatics or individuals with respiratory disorders might respond at lower concentrations, as suggested by the work of Jappinen et al. (1990). In that case, the symptoms were mild and transient; however severe asthmatics were excluded from the study, the concentration tested was low (i.e., 2 ppm) and the duration of exposure was short (i.e., 30 minutes).

Non-clinical studies:
With progressively higher concentrations, the likelihood of adverse clinical symptoms will mount, based largely on the findings from animal studies. However, no consistent evidence was presented to suggest that serious, irreversible symptoms might occur at concentrations up to 100 ppm. The animals generally recovered quickly from any signs of clinical discomfort or stress.

Case-control studies:
Case-control studies consistently revealed a number of subjective complaints allegedly caused by exposure to H_2S among the exposed cohorts. However, these studies received "low" confidence index rankings owing to serious weaknesses in experimental design, conduct and/or reporting

The influence of odour on the likelihood of occurrence of signs and symptoms was not explored as part of the current review. However, in light of reported physiological and/or psychological responses to odours, combined with the unpleasant smell of H_2S and its very low odour threshold, there is a distinct possibility that some individuals might respond at unusually low concentrations.

E. Eye

Despite repeated references in the published literature to the sensitivity of the eye to the irritant effects of H_2S (ACGIH, 1984; ATSDR, 1999; Alberta Health, 1988; Beauchamp *et al.*, 1984; NRCC, 1981) and the earlier claim that *"irreversible eye tissue damage can occur at 20 ppm H_2S ..."* (Alberta Health, 1988), very little evidence of effects on the eye following short-term, "low-dose" exposure surfaced during the current review of the literature.

Clinical studies
Evidence of eye irritation rarely was reported in the controlled clinical studies that were reviewed, albeit a large number of the studies involved exposure to H_2S through a mouthpiece, thereby effectively precluding exposure of the eyes (Bhambhani *et al.*, 1991, 1994, 1996a, 1997b, 1997).

Jappinen *et al.* (1990) found no evidence of eye irritation following controlled "whole body" exposure of asthmatic subjects to 2 ppm of H_2S for 30 minutes (Study ID ◆ 202), nor did Kangas and Savolainen (1987) report eye irritation among subjects exposed to H_2S at concentrations up to 30 ppm for 30 to 45 minutes (Study ID ● 207).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S for as little as 2 to 15 minutes complained of irritation of the eyes. The effects became more pronounced with continued exposure, with *"sharp pain in the* *eyes"* reported after 1 to 4 hours (Study ID ● 444).

Non-clinical studies

With respect to the non-clinical studies, fewer than 10% of the investigations reported eye involvement in response to exposures to H_2S at concentrations between 0 and 100 ppm. The reported effects were typically non-descript. Specifically, Haider *et al.* (1980) reported *"itching and eye irritation"* following "whole-body" exposure of male guinea pigs to 20 ppm of H_2S for one hour per day for 11 days (Study ID ● 179). Kosmider *et al.* (1971) observed *"congestion of the conjunctiva"* among male and female rabbits exposed to 71 ppm of H_2S for one hour per day for 14 days (Study ID ● 595). Lopez *et al.* (1986) reported *"lacrimation"* among male rats exposed to 40 ppm of H_2S for 6 hours (Study ID ● 466). Mitchell and Yant (1925) reported *"irritation of the eyes"* progressing to *"pus in the eyes"* among rats, guinea pigs and dogs following continuous exposure to 35 to 100 ppm of H_2S for 8 to 48 hours. Lacrimation was also noted among the dogs (Study ID ● 444). In each case, the reported effects were not confirmed through *in situ* ophthalmoscopic examination or post-mortem histopathological evaluation of the eyes. It may be of some interest that most of the studies were assigned a "low" confidence index ranking, signaling serious weaknesses in experimental design, conduct and/or reporting. It is also of interest that Skrajny *et al.* (1996) reported that *"eye irritation was never observed in the rats"* as a result of exposure to H_2S at concentrations up to 100 ppm for three hours per day for 5 days (Study ID ● 291). No eye involvement was indicated in any of the remaining non-clinical studies.

Case-control studies

Case-control studies showed mixed results. Kilburn (1997) found that eye effects were not reported by subjects allegedly exposed to 1 to 50 ppm of H_2S for up to 24 hours at the time of exposure; however, upon evaluation two to six years later, eye involvement was reportedly indicated, as evidenced by abnormal colour vision and reduced visual fields as well as complaints of eye irritation (Study ID ● 216). Similarly, Kilburn (1999) reported eye effects among individuals allegedly exposed to H_2S at concentrations ranging from 1 to 40 ppm for up to one week as a result of emissions from a refinery explosion and fire. The effects presented as abnormal color vision and impaired visual fields based on testing performed three years after the event (Study ID ● 217). In addition, witnesses testifying as part of the Lodgepole blow-out inquiry recalled experiencing eye irritation at the time of the incident (Study ID ● 327). Similarly, a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole blow-out revealed a relatively high incidence of eye effects, including "burning eyes" and double vision (Study ID ● 424). As indicated earlier, the confidence index ranking assigned to each of these studies was "low" in light of serious deficiencies in experimental design, conduct and reporting.

It is clear from the above that little information is available suggesting eye involvement following short-term exposure to H_2S at concentrations in the range of 0 to 100 ppm. This finding is in apparent contradiction with the conclusions reached earlier (Alberta Health, 1988). Possible reasons for the discrepancy are outlined later (Chapter VI. Other Considerations).

Summary:

It would appear that very little reliable information exists in the literature concerning the effects of short-term exposure to H_2S at low concentrations on the eye and associated structures.

Clinical studies:

No evidence of eye involvement was indicated as part of the findings from clinical studies involving the controlled exposure of subjects to H_2S at concentrations up to 10 ppm for 30 minutes. However, for most of these studies, the H_2S was delivered via a mouthpiece, thereby effectively eliminating any exposure of the eyes.

Non-clinical studies:

Mixed results were obtained from non-clinical studies. Of the reliable studies, one investigation showed no signs of eye involvement at a concentration of 100 ppm after repeated subacute exposure, while another study reported irritation (as evidenced by *lacrimation) following a single exposure to 40 ppm of H_2S.*

Case-control studies:

Human experience following incidents involving the accidental release of H_2S at low concentrations suggests eye irritation may occur, but the reliability of the information is questionable.

F. Respiratory System

The effects of short-term exposure to H_2S on the respiratory system presented as a spectrum of signs and symptoms as well as functional, structural, and biochemical changes that were consistent with the irritant properties of the gas. These changes are highlighted below.

Signs and Symptoms

Reports of respiratory discomfort or distress following short-term exposure to H_2S at concentrations up to 100 ppm were limited.

Clinical studies

Clinical signs of respiratory involvement were absent in most of the controlled clinical investigations.

Bhambhani *et al.* (1991, 1994, 1996a, 1996b, 1997) found no evidence of respiratory comfort or distress among healthy volunteer subjects following exposure to H_2S at concentrations up to 10 ppm for 30 minutes. The lack of signs and symptoms is especially noteworthy since the subjects inhaled the H_2S while performing moderate to vigorous exercise, which would act to augment the dose received.

Jappinen *et al.* (1990) reported that only initial transient dryness of the throat and nasal passages was experienced by asthmatic subjects during exposure to 2 ppm of H_2S for 30 minutes. Although two of the 10 subjects showed functional changes in airway resistance and airway conductance consistent with bronchial obstruction, clinical presentation was normal. Severe asthmatics were excluded from the study (Study ID ◆ 202).

Larsson *et al.* (1994) reported no airway symptoms among volunteer subjects during confinement in a swine containment building containing low levels of H_2S (< 0.05 ppm) for up to five hours (Study ID ● 662).

Mitchell and Yant (1925) observed coughing and respiratory discomfort among male subjects exposed to 100 to 150 ppm of H_2S for as little as 2 to 15 minutes. The work was described as *"preliminary"*,and the study was assigned a "low" confidence index ranking (Study ID ● 444).

Non-clinical studies

Specific references to signs and symptoms showing respiratory involvement were found in only three of the non-clinical studies reviewed. Specifically, Lopez *et al.* (1986) observed moderate respiratory distress among male rats exposed to 40 ppm of H_2S for 6 hours. The symptoms persisted for up to two hours post-exposure. Rats exposed to 300 ppm of H_2S in the same study showed severe signs of distress (Study ID ◆ 466). Kosmider *et al.* (1971) reported *"quicker breathing"* among rabbits of both sexes exposed to 71 ppm of H_2S for one hour per day for 14 days (Study ID ● 595). Finally, Mitchell and Yant (1925) observed signs of laboured breathing among rats and guinea pigs after 8 to 18 hours of continuous exposure to 100 to 140 ppm of H_2S (Study ID ● 444).

Case-control studies

Case-control studies provided little useful information. Kilburn (1997) reported no immediate signs of respiratory discomfort or distress among subjects exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational incidents. However, the same subjects reportedly complained of a number of respiratory symptoms when tested using a self-administered questionnaire two to six years following the events. The symptoms included shortness of breath, dry cough, cough with mucous, cough with blood, dryness of the mouth, and throat irritation. The study was assigned a "low" confidence index ranking owing to serious deficiencies in experimental design, conduct and reporting (Study ID ● 216). Witnesses testifying at the Lodgepole blow-out inquiry a year and a half after the event (1984) recalled experiencing a number of upper and lower respiratory tract symptoms, including sore throat, nasal irritation, nosebleeds, pain on deep breathing and/or shortness of breath. Asthma and emphysema also were reportedly aggravated. Levels of H_2S measured in the vicinity of the well-site ranged from 0.1 to 15 ppm, with the lower concentrations (*i.e.*, < 1 ppm) predominating. The study also received a "low" confidence index ranking (Study ID ● 327). A retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole blow-out showed a high incidence of "respiratory problems", often accompanied by sore throat and cough. The highest frequency of complaints were registered in areas in which "peak" concentrations of H_2S ranged from 0.5 to 5 ppm, with mean daily average concentrations ranging from 0.02 to 0.4 ppm. The study received a "low-to-moderate" confidence index ranking (Study ID ● 424).

Functional

Clinical studies

Investigation of the effects of short-term exposure to H_2S on pulmonary function was generally confined to the clinical studies involving exposures under controlled conditions. Notable in this regard was the series of investigations completed by Bhambhani *et al.* (1991, 1994, 1996a, 1996b, 1997) involving the assessment of changes in physiological, metabolic and biochemical indices of aerobic and anaerobic metabolism in response to H_2S exposure. The studies typically involved brief exposures of young, healthy subjects to H_2S while exercising. Concentrations ranged from 0.5 to 10 ppm, and exposure times varied from 15 to 30 minutes. In all cases, the H_2S was delivered by breathing through a specially-fitted mouthpiece. Assessment of pulmonary function included measurement of ventilation rate, O_2 uptake, CO_2 output, forced vital capacity (FVC), forced expiratory volume (FEV_{1sec}), forced expiratory flow ($FEF_{25\text{-}75\%}$), and/or peak expiratory flow (PEFR), and calculation of respiratory exchange ratio (RER). Exposure-related changes in pulmonary function were generally absent. Although modest shifts in some parameters were reported in some instances, the differences from control exposures (0 ppm) typically were less than 10 percent, and only rarely achieved statistical significance. The most frequent change was a trend toward increased oxygen uptake, especially under high exercise intensity. The changes were not uniformly consistent across subjects, sexes, and studies, signaling that the effects due to exposure, if any, were marginal (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121, Study ID ▲ 122).

Pulmonary function also was assessed by Jappinen *et al.* (1990) among male and female asthmatic subjects exposed to 2 ppm of H_2S for 30 minutes under controlled conditions in a "whole-body" exposure chamber. No changes in FVC, FEV_{1sec}, or $FEF_{25\text{-}75\%}$ were recorded, nor were significant effects on airway resistance and airway conductance observed. However, airway resistance was reported to be increased on average by 26%, and airway conductance decreased on average by 8%, with considerable variability between subjects. For two of the 10 subjects, changes in airway resistance and conductance were both greater than 30%, signaling bronchial obstruction at the functional level. None of the subjects complained of any airway symptoms. The findings were not segregated according to the severity of the subjects' asthma, but severe asthmatics were excluded from the study. The authors concluded that *"exposure for a relatively short time to hydrogen sulphide concentrations appreciably higher than those existing in ambient air do not cause noticeable effects on respiratory function"* (Study ID ◆ 202).

Non-clinical studies

Pulmonary function was assessed in one of the non-clinical studies. Hulbert *et al.* (1989) observed changes in pulmonary function parameters among guinea pigs exposed to 100 ppm of H_2S for one hour. Exposure reportedly produced a transient 10-fold decrease in airway resistance and a corresponding increase in dynamic compliance, which, according to the study investigators, signaled *"an acute bronchodilatory effect"*. Modest changes in tidal volume and tidal frequency also were recorded. The nature of the overall response was unusual and unexpected since the more common response following H_2S exposure is bronchial obstruction. The confidence index ranking assigned to the study was "low" in light of a number of serious weaknesses in experimental design, execution and reporting, including failure to even report the number of animals on test. The clinical relevance of the findings was difficult to assess since the experiment was designed *" to simulate a mouth-breathing condition "*, wherein exposure involved intra-tracheal treatment of the guinea pigs and use of mechanical ventilation to assist breathing (Study ID ● 460).

Case-control studies
Changes in pulmonary function were not specifically assessed as part of the case-control studies that were reviewed.

Structural

Clinical studies
Assessment of changes to the respiratory tract at the structural level did not form part of the clinical studies that were reviewed.

Non-clinical studies
Changes in the morphology and structural integrity of the respiratory tract at either the gross or microscopic level following short-term exposure to H_2S at concentrations in the range of 0 to 100 ppm were noted in several of the non-clinical studies.

At the macroscopic level, changes in the respiratory tract were mixed depending largely on the status of the animal when presented to necropsy. Weedon *et al.* (1940) reported deep red discoloration of the lungs accompanied by massive hemorrhage among mice that died on test following exposure to 63 ppm of H_2S for up to 16 hours. The lungs of a single rat that died on test under the same exposure conditions were less markedly affected, with only some evidence of hemorrhage. The lungs of animals exposed to higher concentrations of H_2S (> 250 ppm) were more severely affected, with massive hemorrhages covering all lobes. Macroscopic findings from the rats and mice that survived exposure (including animals exposed to 16 ppm of H_2S) were unremarkable (Study ID ◆ 316).

Lopez *et al.* (1986) observed no macroscopic lesions among male rats exposed to 40 ppm of H_2S for 6 hours when sacrificed at 0, 18 or 42 hours post-exposure. However, among rats that died following exposure to 300 ppm of H_2S, the lungs were reportedly congested and hemorrhagic, with froth also detected in the upper airways (Study ID ◆ 466). Curtis *et al.* (1975) found no macroscopic evidence of injury to the respiratory tract of pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416).

At the microscopic level, changes to the morphological architecture of the nasal passages and lungs were described by Lopez *et al.* (1986, 1987, 1988a, 1988b) in a series of studies involving "whole body" exposure of male rats to concentrations of H_2S ranging from 10 to 440 ppm for up to six hours. Examination extended to the ultra-structural level in some cases. At concentrations less than 100 ppm, changes detected by light microscopy were mild and generally transient, with involvement of the nasal passages being most pronounced. The changes included: i) the isolated occurrence of only sporadic "background" nasal lesions in rats exposed to 10 ppm of H_2S for four hours (Study ID ◆ 233); ii) mild to severe necrosis of the nasal epithelium, with or without evidence of mild pulmonary oedema among rats exposed to 40 ppm for six hours (Study ID ◆ 466); and, iii) evidence of mild perivascular oedema in rats exposed to 83 ppm for four hours (Study ID ◆ 232). In all cases, the appearance of oedema was transient, with signs clearing within several hours post-exposure. The damage to the nasal epithelium was largely localized to the lateral aspects of the intermediate sections of the passages. Evidence of repair was observed within a few days post-exposure. The changes were more progressive and severe at the higher exposure concentrations (> 200 ppm). At the ultra-structural level, the only change recorded at the lower exposure concentrations was evidence of mild transient distension of the perivascular space among the rats exposed to 83 ppm for four hours (Study ID ◆ 232). As part of a separate investigation, Hulbert *et al.* (1989) found no microscopic changes to the respiratory tract of guinea pigs following a single exposure to 100 ppm of H_2S for one hour (Study ID ● 460).

Case-control studies
Assessment of changes to the respiratory tract at the structural level did not form part of the case-control studies that were reviewed.

Biochemical

Clinical studies
No cytological, enzymatic or biochemical markers within the respiratory tract were monitored as part of the clinical studies that were reviewed.

Non-clinical studies
A number of non-clinical studies were devoted to the examination of the effects of H_2S exposure on biochemical indices, primarily in an attempt to increase understanding of the mechanism of action. Since the respiratory tract represents the most significant portal of entry for H_2S, changes in these biochemical indices within the respiratory tissues was of interest to several investigators. Emphasis was most often directed at examining the effects of exposure on the activity of the respiratory chain enzymes (*e.g.*, cytochrome oxidase) at different levels within the respiratory tree.

Khan *et al.* (1990) reported that "whole body" exposure of male rats to H_2S at concentrations ranging from 10 to 700 ppm for four hours produced dose-related changes in the activities of several pulmonary mitochondrial enzyme complexes associated with oxidative metabolism and cellular energy production. Changes were confined largely to high exposure concentrations (> 200 ppm), both in terms of the number of enzyme complexes affected and the severity of the effects. No changes were observed at an exposure concentration of 10 ppm. At 50 ppm, effects were limited to a moderate reduction in cytochrome *c* oxidase activity only. At higher concentrations, marked inhibition of cytochrome *c* oxidase and succinate oxidase was reported. At the higher exposure concentrations (> 200 ppm), correlation between the inhibition of cytochrome *c* oxidase within the lung tissue and signs and symptoms of intoxication was evident. However, despite moderate to marked inhibition of enzyme activity at 50 to 200 ppm, no obvious clinical evidence of toxicity was presented (Study ID ◆ 210).

In a more recent investigation "nose-only" exposure of male rats to H_2S at concentrations ranging from 10 to 400 ppm on an "acute" (*i.e.*, 3 hours) or "subacute" (*i.e.*, 3 hours per day for 5 days) basis resulted in biochemical changes in the nasal passages and lungs, as evidenced by shifts in tissue sulphide content, cytochrome oxidase activity and the concentrations of sulphide metabolites (Dorman *et al.*, 2002). Changes were reportedly most pronounced in the nasal olfactory epithelium and lung, with lesser effects noted in the nasal respiratory epithelium. In most instances, the nature, time-course and dose-responsiveness of the changes were consistent with the widely-accepted mechanism of action of H_2S, with the effects on cytochrome oxidase activity and tissue sulfide levels within the lung being inversely related. Inhibition of cytochrome oxidase was evident following exposure to H_2S at concentrations as low as 30 ppm. Changes were not evident at 10 ppm. Recovery from the changes was rapid, with certain of the biochemical indices returning to pre-exposure values within one hour after treatment. Regrettably, signs and symptoms were not monitored as part of the study to assist in the interpretation of the clinical relevance of the findings (Study ID ◆ 151).

Lopez *et al.* (1987) examined the early injury and inflammatory response within the respiratory tract of male rats exposed to H_2S through analysis of a series of cellular and enzymatic markers found in nasal and bronchoalveolar lavage fluids. "Whole body" exposures were performed for four hours at concentrations of 10, 200 or 400 ppm. The cellular and biochemical indices monitored included fluid composition, protein content, lactate dehydrogenase activity, alkaline phosphatase activity and gamma-glutamyl transpeptidase activity. Apart from a transient increase in total cell count within the nasal lavage fluid, no changes in the indices were reported at 10 ppm. The change in cell count had resolved within 20 hours post-exposure, demonstrating *"the remarkable reparative capacity of the respiratory epithelium"*. Notable changes to the biochemical and cytological markers were generally confined to the animals exposed to 400 ppm. The responses were consistent with the known irritant properties of H_2S, and presented as cytotoxic effects on the nasal passages and oedematogenic effects within the lung. No symptomatic correlates of the changes were evident as the animals showed no signs of respiratory discomfort or distress (Study ID ◆ 231).

Case-control studies
No cytological, enzymatic or biochemical markers within the respiratory tract were monitored as part of the case-control studies that were reviewed.

Other

A limited number of non-clinical studies were aimed at understanding the potential effects of H_2S exposure on the anti-bacterial defense system of the lung given reports of secondary pneumonia occurring subsequent to accidental poisoning from the gas (*e.g.*, Kemper, 1966; Donham, 1984).

Rogers and Ferin (1981) followed the inactivation of *Staphylococcus epidermidis* delivered via an aerosol challenge to male rats immediately following "whole body" exposure to 45 ppm of H_2S for up to six hours. Exposure for two hours resulted in no changes in the antibacterial defense system of the exposed rats, with the number of colony forming units (CFUs) not differing from control values. However, after 4 and 6 hours of exposure, the inactivation of *S. epidermidis* was significantly reduced (by up to 50-fold). The exact mechanism by which H_2S exposure effected the change was not determined, but the authors postulated that it could be related to altered macrophage function (Study ID ◆ 269).

In a more recent study, Khan *et al.* (1991) examined the viability of pulmonary alveolar macrophages harvested from male rats immediately following exposure to H_2S at concentrations ranging from 50 to 400 ppm for four hours. No significant changes in the viability of the cells were detected at the lower exposure concentrations ($\leq$ 200 ppm). At 400 ppm, a significant decrease in viability was recorded (Study ID ◆ 211).

Summary:
The effects of short-term exposure to H_2S at concentrations up to 100 ppm on the respiratory system have been well studied. Effects have been examined at the symptomatic, functional, structural and biochemical level.

Clinical studies:
Evidence from clinical studies involving controlled exposures of humans suggests that clinical indications of respiratory discomfort are unlikely and that pulmonary function will remain normal among healthy individuals following brief exposures to concentrations up to 10 ppm. The evidence is particularly compelling since the subjects in these studies inhaled the gas directly through a mouthpiece while performing moderate to strenuous exercise. Asthmatics and individuals with other respiratory disorders may be more responsive, as suggested by the work of Jappinen et al. Specifically, although no signs of respiratory discomfort or distress and no impairment of pulmonary function on average were found among the 10 asthmatic subjects, two of the individuals showed functional changes consistent with bronchial obstruction. The latter finding is significant considering that exposure was relatively brief (i.e., 30 minutes), the concentration was low (i.e., 2 ppm), responses were highly variable between subjects, and severe asthmatics were excluded from the study.

Non-clinical studies:
At the structural level, the work of Lopez et al. was particularly revealing, and suggested that changes to the morphology of the respiratory tract following short-term exposure to low concentrations of H_2S are generally mild and reversible, but progress with increasing concentration. No tissue injury was found following exposure of rats for four hours to 10 ppm of H_2S, whereas damage to the nasal epithelium was evident at concentrations as low as 40 ppm, and mild pulmonary oedema was evident at 80 ppm. Recovery from the damage

was rapid, with evidence of repair observed within several hours post-exposure. Interpretation of the clinical relevance of these findings should respect the fact that the rat is an obligate nasal breather. Accordingly, the observed damage to the lining of the nasal passages may have been exaggerated compared to the expected human condition. It is of interest that the reported structural changes correlated well with the reported changes in biochemical and enzymatic markers. A similar pattern of response was evident, with few, if any, "biochemical" effects found following acute or subacute exposure of rats to 10 ppm of H_2S. Moderate inhibition of cytochrome oxidase activity was reported at concentrations as low as 30 ppm. Additional changes in biochemical and enzymatic indices were found as the concentration increased, possibly signaling early evidence of cytotoxic damage. Again, recovery was rapid, with a return to pre-exposure values within as little as one hour after treatment.

The work of Rogers and Ferin suggested that the pulmonary anti-bacterial defense system could be impaired by a relatively brief exposure to a low concentration of H_2S (i.e., 45 ppm). The clinical relevance of this finding is not fully understood; however, it might explain the frequent occurrence of secondary pneumonia reported to occur after accidental poisoning with H_2S in which pulmonary oedema is manifest.

Case-control studies:
Case-control studies provided little reliable information with respect to symptomatic, functional, structural or biochemical effects of short-term exposure of H_2S at concentrations up to 100 ppm on the respiratory system. Signs and symptoms consistent with respiratory involvement were reported (e.g., cough, sore throat, nasal irritation) but confidence in the study findings was low given the weaknesses in experimental design, conduct and reporting.

G. Skin

Very few studies reported changes to the skin and/or integumentary system as a result of short-term exposure to H_2S at concentrations up to 100 ppm.

Clinical studies
Assessment of changes to the skin did not form part of any of the clinical studies reviewed.

Non-clinical studies
Weedon *et al.* (1940) observed preening of the facial area among rats and mice exposed to H_2S for up to 16 hours under controlled conditions. The preening was suggestive of possible irritation of the skin and mucous membranes. However, the response was only observed at exposure concentrations of 250 ppm and higher. No preening or grooming of the skin was evident among the animals exposed to 16 or 63 ppm of H_2S (Study ID ◆ 316). Similarly, Mitchell and Yant (1925) observed *"washing of the face"* and/or *"rough hair"* among rats and guinea pigs following exposure to 35 to 100 ppm of H_2S, possibly suggestive of skin irritation (Study ID ● 444).

Case-control studies
Kilburn (1997) found that subjects reportedly exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational incidents, complained of skin irritation and fingernail changes when completing a self-administered questionnaire 2 to 6 years after the exposure events. The study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting. The deficiencies included use of a very limited sample size (*i.e.,* only 6 subjects were evaluated), possible subject bias in the reporting of complaints, unreliable exposure estimates, and excessive lag period between the reported exposures and the testing of the subjects (Study ID ● 216).

As part of a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole sour gas well blow-out, certain effects on the skin and integumentary system were identified. The effects were described as "skin allergies", loss of hair and ruptured septum. However, the reliability of the findings is questionable since the study suffered from several weaknesses in design, conduct and reporting (Study ID ● 424).

Summary:
Clinical studies:
Assessment of changes to the skin did not form part of any of the clinical studies reviewed.

Non-clinical studies:
No reliable evidence was found to suggest that short-term exposure to H_2S at concentrations up to 100 ppm has an effect upon the skin or integumentary system.

Case-control studies:
Complaints of skin effects have been reported among subjects exposed to low levels of H_2S following accidental releases, but the evidence is largely circumstantial and the reliability of the findings is questionable.

H. Olfactory System

Very few reports showing effects of short-term exposure to H_2S on the olfactory system (*i.e.*, sense of smell) surfaced from the literature reviewed. Although it has been widely reported that high concentrations of H_2S (>150 ppm) can produce olfactory paralysis, no convincing evidence was presented that concentrations up to 100 ppm can elicit the same effect. However, evidence did suggest that olfactory fatigue can occur with concentrations up to 100 ppm.

Clinical studies
Jappinen *et al.* (1990) reported that male and female asthmatic subjects complained of an unpleasant odour immediately following "whole body" exposure to H_2S at a concentration of 2 ppm for 30 minutes. All subjects detected the odour, but rapidly became accustomed to it, presumably due to olfactory fatigue. The response was judged to be distinct from olfactory paralysis which has been documented to occur at significantly higher concentrations of H_2S (*i.e.*, >150 ppm). Several of the subjects also complained of headache following the exposure. (Study ID ◆ 202).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S complained of a loss of the sense of smell within as little as 2 to 15 minutes of commencement of exposure (Study ID ● 444).

Non-clinical studies
At the structural level, evidence suggests that the nasal olfactory epithelium may be especially responsive to H_2S, albeit effects were only reported following exposure of rats to concentrations greater than 100 ppm. Specifically, Lopez *et al.* (1988) reported necrosis and disruption of the cellular architecture of the olfactory nasal epithelium among rats exposed to 400 ppm of H_2S for four hours. Recovery from the damage was slow, with evidence of cellular degeneration still present at 4 days post-exposure. The effects on the olfactory epithelium were much more pronounced than those recorded for the respiratory nasal epithelium (Study ID ◆ 233).

At the cellular level, Dorman *et al.* (2002) also reported the olfactory epithelium to be highly responsive to H_2S. Significant inhibition of cytochrome oxidase within the olfactory nasal epithelium was found among rats exposed to H_2S at concentrations of 80 ppm and higher on either an acute or subacute basis. The response in the olfactory epithelium was greater than that found in the respiratory nasal epithelium. According to the authors, the finding was not unexpected *"as neurons are known to be exquisitely sensitive to chemical-induced hypoxic damage"* (Study ID ◆ 151).

Case-control studies
Kilburn (1997) reported that subjects exposed for "hours" to H_2S at concentrations between 1 and 50 ppm complained of a decreased sense of smell when completing a self-administered questionnaire 2 to 6 years after the exposure. As already indicated, confidence in the study findings was rated "low" in light of serious deficiencies in experimental design, conduct and reporting (Study ID ● 216).

Loss of the sense of smell was reported very infrequently as part of a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole well blow-out. Of several hundred complaints registered following the incident, only one related to the loss, or the sense, of smell (Study ID ● 424).

Summary:
Consistent with earlier reports (Leonardos et al. 1969; Ruth et al., 1986; Amoore and Hautala, 1983), the present review revealed that the odour of H_2S can be detected at low concentrations. Assessment of the physiological and/or psychological responses to the odour of H_2S was not performed as part of the present review. Accordingly, it cannot be determined whether responses occurring secondary to the smell of the gas might present at concentrations up to 100 ppm.

Clinical studies:
In the only reliable study in which reference was made to the smell of the gas, it appeared that the response was transient, and the subjects quickly became accustomed to the odour (i.e., Jappinen et al.). However, the concentration tested was low (i.e. 2 ppm) and the exposure was relatively brief. The subjective response to higher concentrations could not be determined.

Non-clinical studies:
Evidence suggests that cytotoxic injury to the olfactory epithelium may occur at concentrations as low as 80 ppm, thereby introducing the possibility of olfactory paralysis at concentrations approaching 100 ppm. Caution should be exercised when interpreting the clinical relevance of the latter finding since the information relies only on testing with rats, which are obligate nasal breathers and possess a unique nasal architecture.

Case-control studies:
Case control studies revealed only very limited evidence of effects of short-term exposure to low levels of H_2S on the olfactory system. Some indication of impaired sense of smell following exposure was presented. However, confidence in the study findings was rated "low" in light of numerous weaknesses in experimental design, conduct and reporting.

I. Cardiovascular System

Given the high energy and oxygen demands of the heart muscle, together with the interference in tissue oxygen utilization and energy production known to be caused by H_2S, several studies were identified in the published literature in which the effects of short-term exposure to H_2S at concentrations between 0 and 100 ppm on cardiac function were examined. All study types were represented (*i.e.*, clinical, non-clinical and case-control). Unfortunately, much of the information was judged to be unreliable and/or of questionable relevance.

Clinical studies
In a series of clinical trials involving exposure of male and female subjects to H_2S at concentrations ranging from 0.5 to 10 ppm for up to 30 minutes while performing moderate to strenuous exercise, Bhambhani *et al.* (1991, 1994, 1997) reported no significant changes in heart rate, systolic blood pressure, diastolic blood pressure, or rate pressure product. The consistent lack of response in each parameter led the authors to conclude that *"men and women with cardiovascular disease who may be exposed to H_2S due to the nature of their occupations or otherwise, may not be at an increased risk in such an environment"*. Despite the conclusion, the evidence for the absence of cardiac effects is weak since heart rate and blood pressure are imprecise indicators of cardiac toxicity (Study ID ▲ 119, Study ID ▲ 120, Study ID ▲122).

Non-clinical studies
Reference to changes in cardiac function were found in two non-clinical studies, both of which involved exposure of male and female rabbits to H_2S on either an "acute" or "subacute" basis (Kosmider *et al.* 1967, 1971). In the earlier study, exposure of the rabbits to 71 ppm of H_2S on either a single occasion (*i.e.*, 1.5 hours) or repeatedly (*i.e.*, 30 minutes per day for 5 days) reportedly caused cardiac irregularities, as evidenced by changes in ECG tracings and by inhibition of myocardial enzymes. The changes in ECG readings signaled interference in ventricular repolarization as well as the appearance of arrhythmias in the form of ventricular extrasystoles. Recovery occurred within several

days post-exposure. Heart rate was alternately slowed or increased, with no consistent response between animals (Study ID ● 223). In the later study, rabbits were exposed to 71 ppm of H_2S for one hour per day for 14 days. The only reference to cardiac function was a reported increase in pulse rate (Study ID ● 595). Both studies were assigned a "low" confidence index ranking in light of deficiencies in experimental design, conduct and reporting. The deficiencies included inadequate description of the exposure chamber and gas delivery system, failure to analytically confirm the target exposure concentration, inadequate description of exposure conditions, use of a single exposure concentration only, and general lack of control data, especially in relation to the ECG tracings.

Case-control studies
As part of a case-control study to examine the effects of H_2S exposure on neurobehavioral function, Kilburn (1997) found that subjects exposed to concentrations of H_2S ranging from 1 to 50 ppm for "hours" complained of chest tightness and palpitations when completing a self-administered questionnaire 2 to 6 years following exposure. One of the 6 subjects evaluated also recalled experiencing pain in the chest and back at the time of the exposure. The complaints are suggestive of cardiac involvement. However, the study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting (Study ID ● 216).

Several signs and symptoms suggestive of cardiac involvement were recorded as part of a retrospective analysis of the nature and frequency of complaints associated with the Lodgepole sour gas well blow-out. The symptoms included chest pain, suspected heart attack, and high blood pressure. Confidence in the study findings was rated low-to-moderate in light of a number of weaknesses in design, conduct and reporting (Study ID ● 424).

Summary:
Little reliable and/or relevant information exists on the effects of short-term exposure to low concentrations of H_2S on the cardiovascular system.

Clinical studies:
The work of Bhambhani et al. consistently showed no effects following brief exposures to H_2S at concentrations up to 10 ppm on heart rate and blood pressure; however, these measures are relatively imprecise and not overly reliable in assessing cardiac toxicity. Moreover, the subjects who participated in the studies were young, healthy and aerobically fit, and therefore, may not have been representative of the general population. On the other hand, the measurements were performed while the subjects inhaled the H_2S directly through a mouthpiece while exercising at moderate to high intensity, thereby adding considerably to the inhaled dose of the gas compared to that which might be received under normal resting conditions.

Non-clinical studies:
The non-clinical evidence suggested cardiac irregularities in response to short-term exposure to low concentrations of H_2S; however, the studies were weak in terms of experimental design, conduct and reporting, and reliance should not be placed on the findings.

Case-control studies:
Case-control studies revealed evidence suggestive of cardiac involvement following short-term exposure to low levels of H_2S (e.g., chest pain, chest tightness, palpitations); however, the symptoms were non-specific and weaknesses in experimental design, conduct and reporting undermined confidence in the study findings.

J. Liver

Very few studies aimed at examining the potential hepatic effects associated with short-term exposure to H_2S at concentrations up to 100 ppm were discovered in the published literature.

Clinical studies
Assessment of hepatic effects did not form part of any of the clinical studies reviewed.

Non-clinical studies
Kosmider *et al.* (1971) reported that exposure of male and female rabbits to 71 ppm of H_2S for one

hour per day for 14 days caused liver injury, as evidenced by changes in serum glutamic oxaloacetate transaminase activity (SGOT) and serum glutamic pyruvate activity (SGPT). Although the authors claimed that the activity of both enzymes increased in response to exposure (*i.e.*, indicating possible liver damage), careful review of the study findings revealed the SGPT activity actually declined. The lack of consistency in the response of these two well-recognized enzymatic markers of liver integrity hindered interpretation of the toxicological significance of the findings. Histochemical analyses of the liver tissue also revealed changes in the activities of several metallo-enzymes; however, the responses differed among the individual rabbits, and the differences from control values did not achieve statistical significance. The study was assigned a "low" confidence index ranking owing to a number of significant deficiencies in experimental design, conduct and reporting (Study ID ● 595).

Dorman *et al.* (2002) reported changes in cytochrome oxidase activity and sulphide concentrations in liver samples taken from male rats following a single 3-hour exposure to H_2S at concentrations between 10 and 400 ppm. The changes in cytochrome oxidase activity applied to all exposed groups; however, a dose-related pattern was not evident and enzyme activity was *increased* rather than decreased. The increased activity was unexpected, and the authors reported that the biological significance of the change was *"unclear"*. Tissue sulphide concentrations were increased following exposure; however, the changes again showed no obvious dose-related pattern, and differences from control values achieved statistical significance at the higher exposure concentrations only (*i.e.*, $\geq$ 200 ppm) (Study ID ▲151).

Weedon *et al.* (1940) reported discolouration and/or enlargement of the liver as part of the necropsy findings from rats and mice which died on test following a single exposure to 63 ppm of H_2S lasting up to 16 hours. The discolouration was accompanied by congestion in animals found dead after exposure to 250 or 1000 ppm (Study ID ◆ 316).

No reference to hepatic effects was found in the remaining non-clinical studies.

Case-control studies
Assessment of hepatic effects did not form part of any of the case-control studies reviewed.

Summary:
The potential effects of short-term exposure to low concentrations of H_2S on the liver have received little attention.

Clinical studies:
Assessment of hepatic effects did not form part of any of the clinical studies reviewed.

Non-clinical studies:
Possible injury at the cellular level, as evidenced by changes in liver enzyme activities, has been suggested, but the findings are inconclusive. Changes consistent with generalized systemic toxicity have been observed upon gross examination of animals found dead on test following exposure. In both cases, the findings are not especially revealing, nor informative.

Case-control studies:
Assessment of hepatic effects did not form part of any of the case-control studies that were reviewed.

K. Kidney

Even fewer studies aimed at examining the potential renal effects of short-term exposure to H_2S were located in the published literature.

Clinical studies
Assessment of renal effects did not form part of any of the clinical studies reviewed.

Non-clinical studies
Kosmider *et al.* (1971) reported that repeated exposure of rabbits to 71 ppm of H_2S for one hour per day for two weeks caused *"disturbed kidney metabolism"*, as evidenced by changes in the activities of several renal metallo-enzymes determined through histochemical analyses of kidney tissue samples. Interpretation of the biological significance of the observed changes

was hindered by the lack of consistent response between animals and the variable nature of the effects (*i.e.*, some enzymes were inhibited, others were stimulated). Comparison against the control animals was precluded by the lack of reporting of most data (Study ID ● 595).

Weedon *et al.* (1940) reported pale discolouration of the kidneys among rats and mice which died on test following exposure to 63 ppm of H_2S for up to 16 hours. The discolouration was accompanied by congestion among the animals which died after exposure to 250 or 1000 ppm (Study ID ◆ 316).

Case-control studies
Assessment of renal effects did not form part of any of the case-control studies reviewed.

Summary:
Very little information was discovered concerning the potential effects of short-term exposure to low concentrations of H_2S on the kidney.

Clinical studies:
Assessment of renal effects did not form part of any of the clinical studies reviewed.

Non-clinical studies:
Disturbance in kidney "metabolism" as evidenced by changes in the activity of certain renal enzymes was reported, but the data should be considered suspect owing to serious weaknesses in the design, conduct and reporting of the study. The change noted on gross examination of animals which died on test following exposure (i.e., congestion) is non-specific and indicative of generalized systemic toxicity only.

Case-control studies:
Assessment of renal effects did not form part of any of the case-control studies reviewed.

L. Gastrointestinal System

Very few reports of effects of short-term exposure to H_2S at concentrations up to 100 ppm on the gastrointestinal system were found in the literature.

Clinical studies
Gastrointestinal effects were not reported in any of the clinical studies reviewed.

Non-clinical studies
Weedon *et al.* (1940) reported that stomachs from rats and mice that died on test following exposure to 63 ppm of H_2S for up to 16 hours were distended and marked with small hemorrhagic infiltrates when examined at necropsy. The distension was more pronounced among animals that died following exposure to 250 or 1000 ppm (Study ID ◆ 316).

Case-control studies
Complaints of indigestion and swollen stomachs following exposure to H_2S were recorded by individuals as part of a self-reported questionnaire developed by Kilburn (1997). The individuals had reportedly been exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational mishaps, 2 to 6 years earlier (Study ID ● 216).

Witnesses at the Lodgepole blow-out inquiry (1984) testified that they had experienced signs of gastrointestinal upset, such as loss of appetite, nausea and diarrhea at the time of the incident, which they claimed were linked to exposure to H_2S. Concentrations of H_2S measured in the area at the time were routinely less than 1 ppm, but did reach 15 ppm on occasion (Study ID ● 327).

Retrospective analysis of the nature and frequency of health-related complaints registered following the Lodgepole blow-out revealed a number of symptoms consistent with gastrointestinal involvement, including diarrhea, nausea, vomiting, stomach cramps and abdominal pain. However, confidence in the study findings was undermined by several weaknesses in design, conduct and reporting, such as lack of reliable exposure estimates, possible subject bias, and reliance on self-reported symptoms (Study ID ● 424).

Summary:
Little reliable and/or relevant information respecting the effects of short-term exposure to low concentrations of H_2S on the gastrointestinal system was found.

Clinical studies:
No gastrointestinal effects were reported in any of the clinical studies reviewed.

Non-clinical studies:
The findings recorded from the necropsy of animals that died on test following exposure to H_2S are non-specific and cannot be assigned conclusively to treatment.

Case-control studies:
The complaints of gastrointestinal upset and dysfunction reported following accidental releases of H_2S are largely circumstantial.

M. Immunological System

No studies *specific* to the effects of short-term exposure to low concentrations of H_2S on the immune system were found. However, reference to immune function was included in some studies.

Clinical studies
Larsson *et al.* (1994) reported that two of 14 volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for up to five hours tested positive for IgE. However, the positive test results were based on a pre-exposure assessment completed two weeks earlier. No indication of any change in the immunological status of these subjects or the other volunteers due to exposure in the building was found (Study ID ● 662).

Non-clinical studies
Rogers and Ferin (1981) found impairment of the pulmonary anti-bacterial defense system among rats exposed to 45 ppm of H_2S for up to six hours, but the changes could not be specifically or conclusively related to disturbances in cell-mediated or humoral immune function (Study ID ◆ 269).

Although Khan *et al.* (1991) reported a reduction in the viability of pulmonary alveolar macrophages isolated from the lungs of male rats exposed to H_2S for four hours, the change was only expressed at a concentration of 400 ppm. Viability was unaffected by exposure to 50 or 200 ppm of H_2S (Study ID ◆ 211).

Case-control studies
Assessment of immunological effects did not form part of any of the case-control studies reviewed.

Summary:
No reliable and/or relevant information concerning the effects of short-term exposure to H_2S at low concentrations on the immunological system was discovered as part of the current literature review.

Clinical studies:
A single clinical study reported positive IgE test results among selected subjects exposed to very low levels of H_2S for up to five hours in a swine confinement building. However, positive tests were unrelated to H_2S exposure.

Non-clinical studies:
Impairment of the pulmonary anti-bacterial defense system among rats exposed to 45 ppm H_2S was reported; however, the mechanism by which this effect was elicited is unknown.

The reported reduction in the viability of pulmonary alveolar macrophages in response to exposure may be of some significance; however, the concentration at which the effect was observed (i.e., 400 ppm) was outside the range of interest.

Case-control studies:
Assessment of immunological effects did not form part of any of the case-control studies that were reviewed.

N. Hematopoietic System

No studies specific to the effects of short-term exposure to H_2S at concentrations up to 100 ppm on the hematopoietic system (*i.e.*, blood elements, bone marrow, spleen) were located. Some generalized findings are listed below.

Clinical studies
Larsson *et al.* (1994) did report a reduction in

leucocyte counts among volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for up to five hours, possibly related to an intense inflammatory reaction in the airways; however, the authors admitted that the responses were unlikely related to H_2S given the low concentration found. It was concluded that the changes were most likely related to the presence of endotoxins and grain dust (Study ID ● 662).

Non-clinical studies
Studies in which routine necropsy was performed on animals which either died on test or survived exposure to low concentrations of H_2S did not suggest any involvement of the spleen. For example, Curtis *et al.* (1975) found no evidence of gross alterations in the *"visceral organs"* of pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416). Similarly, Weedon *et al.* (1940) did not report any gross evidence of injury to the spleen among rats and mice that died on test during exposure to 63 ppm of H_2S for up to 16 hours. Even at higher concentrations (*i.e.*, up to 1000 ppm), the spleen was not involved (Study ID ◆ 316).

Case-control studies
Assessment of hematopoietic effects did not form part of any of the case-control studies reviewed.

Summary:
The potential effects of short-term exposure to low concentrations of H_2S on the hematopoietic system have received little attention.

Clinical studies:
A single clinical study reported a reduction in leucocyte counts in subjects exposed to very low levels of H_2S for up to five hours in a swine confinement building. However, the author attributed the response to the presence of contaminants other than H_2S.

Non-clinical studies:
No specific evidence was discovered to suggest that exposure is or is without effect on the blood elements, bone marrow, spleen or other components of the hematopoietic system.

Case-control studies:
Assessment of hematopoietic effects did not form part of any of the case-control studies that were reviewed.

O. Nervous System

The effects of short-term exposure to H_2S on both the developing and mature nervous system have received considerable attention. The effects present as a spectrum of symptomatic, functional, structural, behavioral, and biochemical changes. Changes specific to the mature system are highlighted below. Changes specific to the developing nervous system are described later (see Reproductive System). In many cases, the biological significance and clinical relevance of the effects could not be established since the studies showed weaknesses in experimental design, conduct and/or reporting that effectively precluded meaningful interpretation of the findings.

Signs and Symptoms

Clinical studies
Clinical responses suggestive of nervous system involvement were mixed, and depended on the nature of the study, the exposure concentration(s) used, and the duration of exposure. With respect to the clinical studies that were subjected to review, clinical signs of nervous system involvement were generally absent.

Bhambhani *et al.* (1991, 1994, 1996a, 1996b, 1997) reported no clinical symptoms among subjects exposed to H_2S via a mouthpiece at concentrations up to 10 ppm over a 15 to 30 minute period while exercising at moderate to strenuous intensity. None of the subjects complained of headache, fatigue, dizziness, disorientation or other indicators of nervous system involvement (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121, Study ID ▲ 122).

Similarly, no evidence of clinical symptoms consistent with nervous system involvement was reported by Kangas and Savolainen (1987) in a study involving "whole body" exposure of volunteer subjects to H_2S at concentrations up to 30 ppm for 30 to 45 minutes (Study ID ● 207).

No signs or symptoms signaling nervous system involvement were reported by Mitchell and Yant (1925) following exposure of male subjects to 100 to 150 ppm of H_2S for up to 8 hours (Study ID ● 444).

Jappinen *et al.* (1990) reported that three of ten asthmatic subjects exposed to 2 ppm of H_2S for 30 minutes complained of headache following exposure. The subjects also complained of an unpleasant odour at the time of exposure. (Study ID ◆ 202).

Larsson *et al.* (1994) reported that volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for 2 to 5 hours complained of malaise and drowsiness, and less frequently of headache and nausea. The symptoms varied by subject. Other chemicals were also measured inside the building including ammonia, carbon dioxide, dust and endotoxins, each of which singly or in combination might have contributed to the symptoms (Study ID ● 662).

Non-clinical studies
With respect to non-clinical studies, short-term exposure to H_2S at concentrations up to 100 ppm range produced mixed responses, ranging from an absence of any signs and symptoms consistent with nervous system involvement (Khan *et al.*, 1990 – Study ID ◆ 210; Lopez *et al.*, 1987 – Study ID ◆ 231; Lopez *et al.*, 1988 – Study ID ◆ 233; O'Donoghue, 1961 – Study ID ● 255), to initial hyperactivity, often followed by lethargy and drowsiness (Haider *et al.*, 1980 – Study ID ● 179; Kosmider *et al.*, 1971 – Study ID ● 595; Lopez *et al.*, 1986 – Study ID ◆ 466; Mitchell and Yant, 1925 - Study ID ● 444; Savolainen *et al.*, 1980 – Study ID ● 280; Weedon *et al.*, 1940 – Study ID ◆ 316), to unconsciousness (Kosmider *et al.*, 1967 – Study ID ● 223).

Case-control studies
In terms of case-control studies, a wide spectrum of signs and symptoms consistent with nervous system involvement have been reported; however, in all cases, the studies were assigned a "low" confidence index ranking owing to serious weaknesses in study design, conduct and reporting that hindered the interpretation of the findings. Kilburn (1997) reported that subjects exposed to 1 to 50 ppm of H_2S for "hours", primarily as a result of occupational incidents, complained of headache, excessive fatigue, syncope, and/or loss of consciousness at the time of the exposure. The same subjects subsequently complained of a variety of symptoms consistent with nervous system involvement (*e.g.*, headache, dizziness, loss of consciousness, extreme fatigue, loss of balance, nausea) as part of a self-administered questionnaire completed 2 to 6 years after the exposure events (Study ID ● 216).

Witnesses testifying at the Lodgepole blow-out inquiry (1984) recalled experiencing headache and nausea at the time of the incident two years earlier. However, in general, few complaints suggestive of nervous system involvement were registered. Levels of H_2S measured in the surrounding area at the time of the blow-out were typically less than 1 ppm (Study ID ● 327).

A retrospective analysis of the nature and frequency of health-related complaints registered following the Lodgepole blow-out did reveal a number of symptoms indicative of nervous system involvement. The symptoms included headache, nausea, loss of appetite, dizziness, insomnia, tiredness, numbness and tingling of the limbs. Exposures occurred over 2 two-week periods, during which time H_2S was released directly into the atmosphere. Concentrations of H_2S measured in areas from which complaints were heard ranged from "peak" values of 0.3 to 5.5 ppm to mean daily average values of 0.01 to 0.4 ppm (Study ID ● 424).

Functional

Clinical studies
Changes in nervous system function were not specifically assessed as part of the clinical studies reviewed.

Non-clinical studies
Only a single study aimed at examining

functional changes in the nervous system following short-term exposure to H_2S at low concentrations was located. Skrajny *et al.* (1996) reported that male rats exposed to H_2S at concentrations ranging from 25 to 100 ppm for three hours per day for 5 days showed changes in EEG activity signaling disturbance in voluntary motor function (*e.g.*, walking, running, rearing). Autonomic motor function (*e.g.*, shivering) was unaffected. Changes were confined to the hippocampal region of the brain. EEG readings in the neocortex remained normal. The biological significance of the changes was unclear, with the authors admitting that further study was needed to better differentiate the responses and to determine the underlying mechanism (Study ID ◆ 291).

Case-control studies
Changes in nervous system function were not specifically assessed as part of the case-control studies that were reviewed.

Structural

Clinical studies
Effects on the structural integrity of the nervous system were not specifically assessed as part of the clinical studies reviewed.

Non-clinical studies
No studies directed specifically at the examination of changes to the nervous system at the structural level following short-term exposure to H_2S at concentrations up to 100 ppm range were found. However, Weedon *et al.* (1940) noted *"congestion"* of the brains of rats and mice that died on test during exposure to 63 ppm of H_2S for up to 16 hours at the time of necropsy (Study ID ◆ 316). Discussion surrounding the effects of H_2S on nervous system development in neonatal rats can be found in Chapter V-Section P: Reproductive system.

Case-control studies
Changes in nervous system structure were not specifically assessed as part of the case-control studies reviewed.

Biochemical

Clinical studies
No cytological, enzymatic or biochemical markers within the nervous system were monitored as part of the clinical studies that were reviewed.

Non-clinical studies
A sizeable number of studies were identified in which a variety of different biochemical markers within brain tissue were measured in response to short-term exposure to H_2S at concentrations within the range of interest (*i.e.*, 0 to 100 ppm). It appeared that in most instances, the studies were aimed primarily at furthering understanding of the mechanism of action of H_2S at the cellular level. The biochemical markers included enzymes, neurotransmitters, RNA, protein, lipids and other cell constituents. It is noteworthy that most of the studies received a "low" confidence index ranking in light of significant deficiencies in experimental design, conduct and/or reporting.

In an early study, Kosmider *et al.* (1967) determined that brain alkaline phosphatase activity was decreased among male and female rabbits following "whole body" exposure to 71 ppm of H_2S for 1.5 hours relative to control values. The finding supported the contention that H_2S has an affinity to bind to metallo-enzymes, disrupting activity. However, interpretation of the biological significance of the change was hindered by the use of only a single exposure concentration (*i.e.*, dose-responsiveness could not be determined) and by the lack of any symptomatic correlates signaling nervous system dysfunction (*i.e.*, no signs or symptoms were reported). A similar decrease in the activity of serum alkaline phosphatase also was observed (Study ID ● 694).

Elovaara *et al.* (1978) reported that "whole body" exposure of female mice to 100 ppm of H_2S for two hours produced alterations in protein metabolism in the brain, as evidenced by a reduction in the rate of leucine incorporation into cerebral protein and myelin, and inhibition of acid proteinase. However, the changes were not accompanied by any shift in the protein content of the brain or myelin. Although the animals were reportedly *"agitated"* at the start of exposure, no other signs and symptoms consistent with nervous system involvement were recorded. Interpretation of the biological significance of the changes was hindered by the use of only one exposure concentration, and a lack of uniformity in the time-course of the response following exposure (Study ID ● 155).

Savolainen *et al.* (1980) discovered changes in a number of metabolic and enzymatic parameters in brain tissue obtained from female mice exposed on a 4-day interval to 100 ppm of H_2S for up to 16 days. The changes included progressive inhibition of cytochrome oxidase, reduction in RNA synthesis, increased superoxide dismutase activity, increased glutathione concentration, and increased activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase. Apart from some initial *'excitement"* at the initiation of treatment, the changes were not accompanied by any signs of intoxication. The biological significance of the changes was unclear. The majority of changes were relatively modest, and the time-course of the responses was highly variable. Dose-responsiveness could not be determined owing to the use of a single exposure concentration only (Study ID ● 280).

Haider *et al.* (1980) found that subacute exposure of male guinea pigs to 20 ppm of H_2S for 11 days caused regional-specific reductions in the total lipid and phospholipid content of brain tissue. The changes were apparent in the brain stem and cerebrum, but not in the cerebellum. Cholesterol levels were unaffected in all regions. Signs and symptoms consistent with nervous system involvement also were reported and included fatigue, somnolence and dizziness. The biological significance of the shifts in lipid content was not stated. Interpretation was hindered by the use of a single exposure concentration only, which precluded assessment of dose-responsiveness (Study ID ● 179).

In a more recent study, Nicholson *et al.* (1998) reported that "whole body" exposure of male rats to 100 ppm of H_2S for three hours per day for 5 days produced increased levels of L-glutamate in the hippocampal region of the brain. The authors postulated that, because of its role as a neurotransmitter, the changes in L-glutamate levels might contribute to the neurotoxic effects of H_2S. The findings should be considered preliminary in nature since even the authors offered that *"further study is warranted"*. Interpretation of the biological significance of the finding is difficult since only a single exposure concentration was used, and no symptomatic nor pathological correlates of the change could be identified (Study ID ◆ 398).

In a study aimed primarily at examining the neurobehavioral effects of short-term exposure to H_2S, Struve *et al.* (2001) found no changes in brain catecholamine levels among male rats following exposure to H_2S at concentrations up to 400 ppm for three hours per day for 5 days. The catecholamines measured included dopamine, serotonin, homovanillic acid, 5-hydroxyindole acetic acid, and 3, 4-dihydroxyphenylacetic acid (Study ID ◆ 296).

Case-control studies
No cytological, enzymatic or biochemical markers within the nervous system were monitored as part of the case-control studies that were reviewed.

Behavioral

Three studies were located in which the effect of short-term exposure to low concentrations of H_2S on neurobehavioral function was assessed. Two of the studies were "case-control" in nature and involved the evaluation of neurobehavioral function in subjects reportedly exposed to H_2S for several hours using a battery of neurophysiological and neuropsychological tests. The remaining study was a non-clinical investigation in which rats were subjected to a series of tests to assess learning and memory as well as spontaneous motor activity following "whole body" or "nose only" exposures to H_2S on a subacute basis.

Clinical studies
Assessment of behavioral changes was not specifically conducted as part of the clinical studies reviewed.

Non-clinical studies

Struve *et al.* (2001) reported that neurobehavioral function of male rats exposed to 10, 30, 80, 200 or 400 ppm of H_2S for three hours per day for 5 days was largely unaffected at the lower dose levels (*i.e.*, < 200 ppm), as evidenced by normal performance in a water maze and no change in fixed interval operant behavior. The findings suggested that memory and learning were unaffected by treatment. The only change noted at 80 ppm was a reduction in spontaneous motor activity following "nose only" exposure. The change was judged to be of questionable significance since only 2 of 10 rats were affected, and the response was not seen following "whole body" exposure at the same concentration. No changes were observed among the animals exposed to 10 or 30 ppm of H_2S (Study ID ● 296).

Case-control studies

Kilburn (1997) reported that subjects exposed to H_2S at concentrations estimated to range between 1 and 50 ppm for "hours" showed deficits in neurobehavioral performance when evaluated 2 to 6 years later. Deficits were reportedly found for balance, choice reaction time, dexterity, coordination, colour discrimination, hearing, and grip strength. Mood also was affected. In addition, subjects complained of memory loss, loss of concentration, irritability, loss of libido and mood swings. The subjects evidently showed no deficits in most measures of cognitive function, including non-verbal non-arithmetical intelligence, integrative capacity, vocabulary and verbal recall. As already indicated, the study was assigned a "low" confidence index ranking in light of serious deficiencies in experimental design, conduct and reporting that essentially precluded meaningful interpretation of the biological significance or clinical relevance of the findings (Study ID ● 216).

In a subsequent study, Kilburn (1999) found that subjects exposed to H_2S at concentrations estimated to range between 1 and 40 ppm for up to several days as a result of emissions from a refinery explosion and fire showed neurobehavioral deficits upon evaluation 3 years later using essentially the same battery of tests. However, the measures affected were not in good agreement with the earlier findings, and inconsistencies in the results were even evident between the two sub-cohorts of subjects included in the study. For instance, unlike the earlier findings, dexterity and coordination appeared to be unaffected, but cognitive function was impaired based on measurement of vocabulary and verbal recall. In addition, conflicting findings were reported for the two exposed sub-cohorts for choice reaction time, balance (eyes closed), verbal recall, long-term memory, dexterity, and coordination. Confidence in the study findings was rated "low" owing to serious weaknesses in design, conduct and reporting. The weaknesses included possible selection bias in the choice of subjects, unreliable exposure estimates, excessive lag period between the time of exposure and subsequent evaluation of subjects, and failure to control for obvious confounding variables (*i.e.*, exposure to other chemicals) (Study ID ● 217).

Summary:

A significant amount of information describing the effects of short-term exposure to H_2S at concentrations up to 100 ppm on the nervous system was discovered. Highlights are necessarily limited to the information obtained from studies which achieved a confidence index ranking of "moderate" or "high". No reliable evidence was found to suggest that short-term exposure to low concentrations of H_2S is associated with neurobehavioral impairment.

Clinical studies:

Clinical signs signaling nervous system involvement are unlikely to be seen following exposure to H_2S at concentrations up to 10 ppm. The work of Bhambhani et al. consistently showed a lack of any signs or symptoms among the test subjects following brief exposure to 0.5, 2, 5 or 10 ppm of H_2S, despite inhaling the gas directly through a mouthpiece while exercising at moderate to high intensity. Complaints of headache surfaced during the work of Jappinen et al.; however, only three of the 10 subjects complained.

Non-clinical studies:

At concentrations above 10 ppm, reliance can only be placed on the findings from non-clinical studies, with obvious limitations. Moreover, these *studies showed mixed results. However, the information from those studies judged to be most reliable revealed no signs or symptoms consistent with nervous system involvement among the test animals even at concentrations up to 100 ppm.*

Information respecting the effects of short-term exposure to low concentrations of H_2S on the nervous system at the functional, structural and "biochemical" level proved difficult to interpret. Although changes in a variety of metabolic, enzymatic and biochemical indices have been reported following exposures to concentrations up to 100 ppm, the toxicological significance and clinical relevance of the findings remain elusive. Part of the difficulty relates to the fact that much of the information comes from studies with serious weaknesses in experimental design, conduct and reporting. Very little, if any, confidence could be placed in the results and conclusions from these studies. Accordingly, the reported changes in protein metabolism, lipid content, RNA synthesis, and enzyme activities in the nervous tissue cannot be assigned biological significance. In addition, even the findings from the studies which achieved a "moderate" confidence index ranking and suggested possible functional changes in the brain (i.e., altered EEG activity) or disturbances in neural transmission (i.e., shifts in L-glutamate levels) could not be assigned toxicological significance owing to a lack of understanding of the mechanisms involved and/or the clinical implications. Even the authors admitted that the results should be considered preliminary and that further study was warranted

Case-control studies:
No reliable evidence of effects of short-term exposures to low levels of H_2S on the nervous system was found. Any evidence that was discovered was largely circumstantial. In addition, the evidence was suspect owing to numerous weaknesses in the design, conduct and reporting of the studies.

P. Reproductive System

It was reported earlier (Alberta Health, 1988) that the reproductive effects of low concentrations of H_2S had received little attention, and that any effects observed needed *" to be corroborated by further studies "*. Since that time several non-clinical studies aimed at examining the reproductive and developmental toxicity of H_2S have been completed. In many instances, the duration of exposure extended over several weeks, with evaluation of reproductive and developmental parameters made during the pre-mating, mating, gestational and post-natal periods. Although the exposure duration was beyond "subacute", the studies were included in the present review in light of the importance of identifying and understanding any potential impacts of H_2S on pregnancy, reproductive outcome, and early development. The inclusion of the studies also respected the potential increased sensitivity of the developing fetus to the effects of H_2S. Various parameters were measured during the course of the studies, and were broadly classified as signs and symptoms, reproductive, structural, developmental and biochemical for review purposes. Confidence index rankings assigned to the studies varied from "high" to "low".

Signs and Symptoms

Clinical studies
No clinical reproductive studies were identified for review.

Non-clinical studies
Despite the widely accepted recommendation that signs and symptoms be routinely monitored and recorded during the course of reproductive and developmental toxicity studies, the results of routine observation of the test animals were rarely reported as part of the non-clinical studies. The exception was a well-designed study by Dorman *et al.* (2000) that followed a conventional study protocol and was performed in compliance with USEPA Good Laboratory Practice (GLP) regulations. No signs and symptoms of toxicity were observed among the parental animals following "whole body" exposure to H_2S at concentrations ranging from 10 to 80 ppm for six hours per day for at least 70 days (*i.e.*, throughout a two-week pre-mating period, a two-week mating period, a three-week gestation period, and a three-week post-natal period). However, both

the male and female parents showed reduced feed intake, accompanied by a slowing of body weight gain following initial exposure to 80 ppm of H_2S, possibly indicative of generalized and/or non-specific toxicity (Study ID ▲ 149).

In a series of studies completed by Roth and co-workers (Roth and Hannah, 1989; Hannah *et al.*, 1990; 1989; Hayden *et al.*, 1990a, 1990b; Hannah and Roth, 1991; Skrajny *et al.*, 1992) exposures evidently were performed in a specially-constructed chamber that allowed for clear observation of the test animals. However, no signs and symptoms were reported. Information respecting the feed intake of the dams was provided in one of the studies of the series and showed reduction in consumption during the initial stage of gestation following exposure to 50 or 75 ppm of H_2S, again suggestive of generalized stress and/or non-specific toxicity (Study ID ● 630, Study ID ●180, Study ID ● 402, Study ID ◆ 564, Study ID ◆ 12, Study ID ● 11, Study ID ◆ 290).

Similarly, Saillenfait *et al.* (1989) failed to report signs and symptoms as part of a study to examine developmental defects following exposure of pregnant rats to H_2S at concentrations up to 150 ppm from Day 6 to Day 20 of gestation, either alone or in combination with carbon disulphide (Study ID ◆ 278).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters did not form part of the case-control studies reviewed.

Reproductive

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Dorman *et al.* (2000) reported no changes in reproductive parameters following exposure of male and female rats to 10, 30 or 80 ppm of H_2S for six hours per day throughout a two-week pre-mating, two-week mating, and three-week gestation period. Mating index, fertility index, post-implantation losses, number of late resorptions, length of gestation, delivery time, number of stillbirths, number of females with live pups, and number of implants per pregnant female were unaffected by exposure (Study ID ▲ 149).

Hayden *et al.* (1990b) found no effects on gestation length, litter size, pup viability and pup sex ratio following exposure of pregnant rats to H_2S at concentrations ranging from 20 to 75 ppm for seven hours per day from Day 6 to Day 21 of gestation. However, the exposed dams reportedly experienced dystocia (*i.e.*, prolonged and difficult delivery), in an apparent dose-related fashion. Careful examination of the data revealed that the dystocia was very much animal-dependent, with many of the dams having a normal length of delivery. The incidence of dystocia among the exposed dams also was within the range reported for the various sets of control dams used in the study. No evidence was provided that the dystocia adversely affected the health of the dams, and apart from a *"few"* fetuses that reportedly may have died from asphyxiation due to the prolonged delivery, the fetuses also were unaffected. The effect was judged to be of questionable significance (Study ID ◆12). No evidence of dystocia was reported by Dorman *et al.* (2000)

Saillenfait *et al.* (1989) exposed pregnant rats to 50, 100 or 150 ppm of H_2S for six hours per day from Day 6 to Day 20 of gestation, either alone or in combination with carbon disulphide. The authors reported that exposure to 50 ppm or 100 ppm of H_2S was without effect on reproductive parameters, including fertility index, number of implantation sites, number of resorptions, and number of live and dead fetuses. No clear adverse effects were even evident at 150 ppm. Some evidence of maternal toxicity at the highest exposure concentration was provided by the observation of reduced maternal body weight gain (Study ID ◆ 278).

Reproductive parameters were not reported in any of the remaining non-clinical studies.

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters was not specifically conducted as part of the case-control studies that were reviewed.

Structural

Regrettably, very few of the studies included reference to the health and appearance of the pups at the time of delivery. Again, this serves to emphasize the fact that many of the studies included in the present review failed to follow conventional testing protocols. As a result, much useful information was "lost", and, in this case, assessment of the potential teratogenic effects of H_2S was hindered.

Clinical studies
No clinical reproductive/developmental toxicity studies were identified for review.

Non-clinical studies
Dorman *et al.* (2000) reported isolated instances of external malformations among pups borne by dams exposed to 10, 30 or 80 ppm of H_2S throughout gestation, but the findings were assigned little significance since the differences from control values were not statistically significant and dose-responsiveness was not evident for any of the structural anomalies (Study ID ▲149).

Saillenfait *et al.* (1989) found no evidence of external, skeletal or soft tissue abnormalities related to treatment among pups borne by dams exposed to 50, 100 or 150 ppm of H_2S from Day 6 to Day 20 of gestation, even in the presence of maternal toxicity at the higher exposure concentrations (Study ID ◆ 278).

The findings suggest that H_2S is not teratogenic.

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters was not specifically conducted as part of the case-control studies reviewed.

Developmental

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Developmental landmarks were monitored in two of the reproductive toxicity studies as indicators of the potential effects of H_2S exposure on the growth and development of the offspring. Hayden *et al.* (1990b) found no change in the elapsed time required for incisor eruption, eyelid opening and development of surface righting reflex among pups exposed *in utero* and postnatally to H_2S at concentrations ranging from 25 to 75 ppm compared to corresponding controls. However, the time required for pinnae detachment and hair development was reportedly altered by H_2S exposure. The biological significance of the latter findings was judged to be questionable since the changes were not uniform, not dose-responsive, and/or not statistically significant. No changes in pup body weights as a result of exposure were reported (Study ID ◆ 12).

Dorman *et al.* (2000) noted that developmental landmarks were unaffected by *in utero* and postnatal exposure of pups to H_2S at concentrations ranging from 10 to 80 ppm, as evidenced by no change in the elapsed time required for pinnae detachment, incisor eruption, negative geotaxis, eyelid opening and vaginal patency or preputial separation compared to controls. In addition, pup body weights were unaffected by exposure (Study ID ▲149).

Case-control studies
Assessment of the effect of H_2S exposure on developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Behavioral

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with

short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
The effects of H_2S exposure on neurobehavioral development were followed in a single study only. Dorman *et al.* (2000) reported that pups exposed *in utero* and postnatally to H_2S at concentrations ranging from 10 to 80 ppm showed no neurobehavioral deficits, as evidenced by normal scores when tested for spontaneous motor activity, acoustic startle response, and passive avoidance. Assessment through a functional observation battery (FOB) also revealed no behavioral effects due to exposure. The assessment included examination for signs of ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration, or abnormal pupil reflex, as well as evaluation of tail pinch response, approach response, visual placing response, hind and forelimb grip strength, hind-limb splay, urine and feces production, rearing behavior, neuromuscular dysfunction, and appearance. All parameters were reported to be unaffected by treatment (Study ID ▲ 149).

Case-control studies
Assessment of the effect of H_2S exposure on neonatal behavior and development was not specifically conducted as part of the case-control studies that were reviewed.

Pathology

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Gross and microscopic examination of the organs and tissues from parental animals and/or offspring formed part of the design of a number of the reproductive toxicity studies, with an emphasis on neuropathology.

Using a conventional study design, Dorman *et al.* (2000) found very few changes in the tissues of parental animals following "whole body" exposure to H_2S at concentrations ranging from 10 to 80 ppm for six hours per day for up to 70 days when assessed grossly and microscopically. Adrenal weights of selected parental males were decreased relative to controls, but the response was not dose-related and was considered unrelated to treatment. Selected dams showed reduced ovarian weights, but again, the response was not dose-related. All remaining organ weights of the parental animals were normal (*i.e.*, liver, kidney, heart, lungs, spleen, brain, testes). Evidence of mild to marked changes in the olfactory mucosa was found upon microscopic examination, but details concerning dose-responsiveness were not available. The finding was not unexpected given the irritant properties of H_2S and the fact that the rat is an obligate nose breather. In addition, some evidence of seminiferous tubular generation was reported among the male rats exposed to 80 ppm of H_2S, but the biological significance of the finding was questionable since the cases were isolated and sperm production was normal. Pathological findings among the offspring were reported to be unremarkable, with no changes in the brain, spinal cord or peripheral nerves noted on microscopic examination. No changes in pup organ weights (*i.e.*, adrenal glands, brain, heart, kidneys, liver, lungs, spleen, testes) due to exposure were found (Study ID ▲149).

As part of a series of studies, Roth and co-workers examined the morphological effects of exposure to low concentrations of H_2S on the developing nervous system of the rat, with particular emphasis on changes to the density and architecture of cerebellar Purkinje cells (Hannah and Roth, 1991; Roth and Hannah, 1989). Pups were exposed *in utero* for seven hours per day from Day 5 to Day 21 of gestation to H_2S at concentrations up to 75 ppm. Increased numbers of Purkinje cells in the cerebellum were discovered following exposure. The architecture of the cells also was reportedly altered, with structural changes evident in the "dendritic tree" (*e.g.*, altered dendritic segment length, terminal path length, segment frequency). The authors offered that the changes were indicative of abnormal development at the "growth front" of the cells, which could pre-dispose the animals to abnormal neurological function. However, it was admitted that interpretation of the findings was *"very difficult"* owing to the complexity of the developing nervous system. The biological significance of the findings was judged to be questionable in light of a lack of dose-responsiveness. Interpretation of the clinical relevance of the findings was hindered by the failure to record any signs and symptoms of toxicity which might have revealed symptomatic correlates of the changes in cellular density and architecture. The studies were assigned a "low" or "low to moderate" confidence index ranking owing to weaknesses in experimental design, conduct and reporting (Study ID ● 11 and Study ID ● 630).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Biochemical

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Roth and co-workers examined a number of biochemical indices as part of a series of investigations aimed at determining the potential developmental effects of H_2S. The indices were assessed in dams and/or offspring following "whole body" exposure to H_2S at concentrations ranging between 20 and 75 ppm, typically for seven hours per day from Day 5 to Day 21 of gestation. The biochemical indices chosen for study allowed for assessment of the potential effects of H_2S on carbohydrate metabolism (*i.e.*, serum glucose), lipid metabolism (*i.e.*, serum and/or tissue triglycerides and cholesterol), protein metabolism (*i.e.*, tissue protein and RNA content), and neural transmission (*i.e.*, serum and/or tissue taurine, glycine, glutamate, aspartate, gamma-aminobutyric acid, serotonin, norepinephrine). Confidence index rankings for the studies were generally marginal (*i.e.*, "low" or "low-to-moderate") in light of weaknesses in experimental design, conduct and/or reporting. The studies did not follow a conventional design, and deviated significantly from the recommendations contained in testing guidelines for developmental toxicity studies.

In an early study in the series, Hannah *et al.* (1989) exposed pregnant rats to 75 ppm of H_2S during much of the gestational period, and reportedly found changes in the levels of selected amino acid neurotransmitters in the brains of the offspring. The authors postulated that the changes might result in long-term behavioral problems; however, it was offered that the consequences of the changes were *"as yet unknown"*, and that the interpretation of the biological significance of the findings *"was very difficult"*. Interpretation was hindered especially by a lack of uniformity in the time-course of the changes and the use of a single exposure concentration only, which precluded any assessment of dose-responsiveness. In addition, the exact role of the amino acids in neural transmission was stated to be unknown. No symptomatic correlates of the changes could be determined owing to a lack of reporting of any signs or symptoms (Study ID ● 180). The findings departed somewhat from those reported by Struve *et al.* (2001) in which no changes in brain catecholamine levels were found among male rats repeatedly exposed to H_2S at concentrations up to 400 ppm. The difference in findings may be age-related since Struve *et al.* used adult animals. In addition, each set of investigators monitored a different battery of amine neurotransmitters.

In a subsequent study, Hannah *et al.* (1990) reported that exposure to 50 ppm of H_2S during Day 6 to Day 21 of gestation resulted in elevated serum taurine levels among the dams both at the time of delivery and weaning of the pups. Although the authors claimed that the elevated maternal taurine levels could have the potential to cause neurological abnormalities in the offspring, the toxicological significance of the finding was unclear. The corresponding levels of taurine in the pups were not monitored, and no signs or symptoms of toxicity were recorded. Dose-responsiveness could not be assessed owing to the use of a single exposure concentration only. The study was assigned a "low-to-moderate" confidence index ranking, signaling weaknesses in experimental design, conduct and reporting (Study ID ● 402).

As part of the same series of studies, Hayden *et al.* (1990a) monitored a number of enzymatic and metabolic indices among the dams and offspring following exposure to H_2S at concentrations ranging from 20 to 75 ppm for seven hours a day throughout gestation. Changes were limited to a dose-related increase in circulating glucose levels and a corresponding decrease in serum triglyceride levels in the dams. The same response was not seen in the pups, albeit serum triglyceride levels were modestly depressed and cholesterol levels were marginally increased. All other parameters were unaffected by exposure (*i.e.*, serum protein level, serum alkaline phosphatase activity, SGOT activity, and SGPT activity). The biological significance of the changes in maternal serum glucose and trigyceride levels is unclear, with even the authors offering that *"further studies will be required"* to understand the basis of the responses (Study ID ◆564).

Hayden *et al.* (1990b) subsequently exposed pregnant rats to 20, 50 or 75 ppm of H_2S during much of gestation and found no changes in a number of biochemical indices among both the dams and offspring. Liver protein levels, liver DNA levels, and liver cholesterol levels were unaffected by exposure. Similarly, brain protein levels, brain DNA levels, and brain cholesterol levels remained unchanged following treatment (Study ID ◆ 12).

In the most recent study of the series, Skrajny *et al.* (1992) monitored monoamine neurotransmitter levels in the brains of pups borne by dams exposed to 20 or 75 ppm of H_2S for seven hours per day from Day 5 to Day 21 of gestation. Treatment reportedly caused shifts in the levels of serotonin and norepinephrine in the developing cerebellum and/or frontal cortex, which the authors postulated could result in permanent structural and neurochemical alterations in the central nervous system. However, interpretation of the findings was confounded by a lack of clear dose-responsiveness in the changes, as well as a highly variable time-course of response for each parameter. No symptomatic correlates of the changes could be determined since no signs or symptoms were reported.(Study ID ◆ 290). The findings contradict those reported by Struve *et al.* (2001).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Other

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
The potential mitogenic effects of H_2S in embryonic tissues were examined by Barilyak and Vasil'eva (1974). Samples of liver and kidney were harvested from pups borne by dams exposed to 7 ppm of H_2S in combination with carbon bisulphide throughout gestation. No significant changes in the mitotic index of the hepatic or renal embryonic tissues were found. The study was assigned a "low" confidence index ranking in light of serious weaknesses in experimental design, conduct and reporting (Study ID ● 497).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Summary:
Clinical studies:
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were

found in the published literature.

Non-clinical studies:
It is regrettable that much of the work did not follow conventional testing protocols, and as a result, much useful information has been "lost" and interpretation of the biological significance of certain of the findings has been hindered. The work of Dorman et al. features prominently since it was performed in accordance with recognized testing guidelines and in compliance with the USEPA Good Laboratory Practice (GLP) regulations. The findings from this work suggest that H_2S *is neither a reproductive toxicant, nor developmental toxicant. Routine measures of reproductive performance were unchanged among the parental animals following repeated daily exposure to* H_2S *at concentrations up to 80 ppm throughout pre-mating, mating and gestational periods. No structural malformations related to treatment were found among the fetuses borne by the exposed dams, suggesting that* H_2S *is non-teratogenic. The results in this respect were supported by the previous work of Saillenfait et al., albeit only a "low" level of confidence could be assigned to the earlier findings. Growth and development of the offspring appeared to be unaffected by treatment, with no changes in survival, body weights, and developmental landmarks resulting from in utero and postnatal exposure. No neurobehavioral deficits were noted among the offspring when evaluated through a battery of tests. Pathology of the parental animals and offspring was generally unremarkable.*

The work of Roth et al. also features prominently; however, interpretation of the biological significance of many of the findings was hindered by the failure to follow conventional testing protocols as well as by weaknesses in study design, conduct and reporting. Apart from these practical difficulties in interpretation, the assessment of the significance of the findings also was hindered by the sheer complexity of the developing nervous system which served as the focus of much of the work. Even the authors admitted that interpretation of much of the work was "very difficult" and that the consequences of certain of the changes were "as yet unknown" and require "further study". Accordingly, although the reported changes in the density and architecture of Purkinje cells, the shifts in amino acid neurotransmitter levels, and the indications of possible disturbances in carbohydrate and lipid metabolism during development may be of some interest, the biological significance and clinical relevance of the findings are unknown.

Case-control studies:
Assessment of the effect of H_2S *exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.*

Q. Metabolic Systems

Study of the potential metabolic effects of short-term exposure to H_2S at concentrations up to 100 ppm has been aimed primarily at identifying and understanding the metabolic and physiologic implications surrounding the inhibition of cytochrome *c* oxidase and the subsequent impairment of oxygen utilization and disruption of energy production at the cellular level. Study has also been directed at examining the metabolic outcomes surrounding the inactivation of metallo-enzymes other than cytochrome oxidase following H_2S exposure.

Clinical studies
Reliance can be placed on a series of studies performed by Bhambhani and co-workers in which a number of physiological, hematological, metabolic and biochemical indices were monitored among male and/or female volunteer subjects during and following exposure to H_2S under strictly controlled conditions (Bhambhani and Singh, 1991; Bhambhani *et al.*, 1994; Bhambhani *et al.*, 1996a; Bhambhani *et al.*, 1996b; Bhambhani *et al.*, 1997). The intent was to explore the presumed shift that would occur from aerobic to anaerobic metabolism following exposure in view of the expected inhibition of cytochrome oxidase and the subsequent disruption of mitochondrial oxidative phosphorylation. Subjects were exposed to H_2S at concentrations ranging from 0.5 to 10 ppm under conditions of moderate to strenuous exercise for 15 to 30 minutes. In all cases, exposure to the gas was via a specially-fitted mouthpiece, thereby precluding any confounding of findings as a

result of possible physiological and/or psychological responses to the odour of H_2S. In most instances, exposures were performed in replicate, adding to the reproducibility of the findings. Subjects were young and generally fit. Confidence index ranking for the entire series of studies was "high" as the investigations were well-designed and well-executed (Study ID ▲118, Study ID ▲119, Study ID ▲ 120, Study ID ▲122).

In the initial study of the series (Bhambhani and Singh, 1991) male subjects were exposed to 0.5, 2 or 5 ppm of H_2S for approximately 15 minutes while performing exercise at three levels of intensity. Pulmonary and cardiovascular indices remained largely unchanged by exposure (*i.e.*, heart rate, oxygen uptake, CO_2 output, ventilation rate), except for a modest increase in oxygen uptake at the highest concentration while exercising at maximum intensity. In terms of metabolic parameters, blood lactate concentrations showed a tendency to increase during exposure, with the change reported to be significant at 5 ppm. The change was consistent with the response that might be expected; however, it was assigned little significance since, even at 5 ppm, the increase in lactate concentration (up to 65%) was well below that resulting from changes in exercise intensity alone (450%). The subjects experienced no discomfort (Study ID ▲ 119).

In a subsequent study aimed at investigating possible differences in physiologic and metabolic responses to H_2S exposure between males and females, subjects were exposed to 5 ppm of H_2S for 30 minutes while exercising at moderate intensity. Exposure was found to be without effect on metabolic, pulmonary, cardiovascular, and arterial blood parameters, irrespective of sex. A numerical, but not statistically significant, increase in blood lactate concentration (~10%) was observed in both sexes. None of the subjects complained of any clinical symptoms. The lack of response among the female subjects was unexpected given the lower blood volumes and hemoglobin content typically reported for the female sex (Study ID ▲ 120).

In a study aimed at investigating the effects of H_2S exposure on the metabolic profile of skeletal muscle (Bhambhani *et al.* 1996a), a series of enzymatic markers of aerobic and anaerobic metabolism was monitored in male and female subjects following exposure to 5 ppm of the gas for 30 minutes while performing moderate exercise. Other than a modest to moderate decrease in the activity of citrate synthase (~20%) among the exposed male subjects, pulmonary, metabolic and enzymatic parameters were reportedly unchanged by treatment. However, careful review of the data also revealed modest shifts in muscle lactate concentration, lactate dehydrogenase activity and cytochrome oxidase activity among the male subjects that were possibly consistent with a shift from aerobic to anaerobic metabolism. No such changes were evident for the female subjects. The clinical presentation of all subjects was unaffected by the exposure (Study ID ▲118).

In the most recent study of the series, the effects of exposure to 10 ppm of H_2S on pulmonary, cardiovascular, metabolic and biochemical parameters were assessed in male and female subjects exposed for 30 minutes while performing moderate exercise (Bhambhani *et al.*, 1997). Results were mixed, with a modest (~ 10%), but unexpected decline in oxygen uptake observed for both sexes, and a modest (~ 11%), but unexpected *increase* in cytochrome oxidase activity noted for the female subjects. In addition, both sexes showed a modest (~ 7%), but unexpected reduction in muscle lactate dehydrogenase activity. These findings argued against any shift from aerobic to anaerobic metabolism as a result of exposure; however, other changes were consistent with such a shift (*i.e.*, increased muscle lactate concentration, reduced citrate synthase activity in both genders). Clinical signs were absent among all subjects. Although the authors concluded that the findings provided cause to question the validity of the current occupational exposure limit (OEL) for H_2S (*i.e.*, 10 ppm), the conclusion was judged to be tenuous given the lack of consistency or responses(Study ID ▲ 122).

Non-clinical studies

Earlier metabolic work performed by Kosmider *et al.* (1971) using rabbits exposed to 71 ppm of H_2S for one hour per day for 14 days showed disturbances in the acid/base balance and mineral status of the blood as well as shifts in the activities of various serum and tissue enzymes that reportedly indicated damage to the liver and impaired kidney function. Interpretation of the significance of the findings was hindered by serious weaknesses in experimental design, conduct and reporting, including inadequate description of the exposure chamber and gas delivery system, use of a single exposure concentration only, failure to include conventional measures of toxicity as part of the overall study design, and failure to properly confirm the target exposure concentration. The study was assigned a "low" confidence index ranking (Study ID ● 595).

Case-control studies

The effects of H_2S exposure on metabolic parameters were not specifically examined as part of the case-control studies reviewed.

Summary:

The results suggest that brief exposures to H_2S at concentrations up to 10 ppm are well tolerated at the "metabolic" level, with some evidence of a possible shift from aerobic metabolism to anaerobic metabolism owing to inhibition of cytochrome oxidase.

Clinical studies:

The "metabolic" work performed by Bhambhani et al. has contributed to a better understanding of the clinical, physiological, and cellular responses to short-term exposures to low concentrations of H_2S. The work was well-designed and well-executed, thereby adding to the significance of the findings. However, at the exposure concentrations tested, the metabolic shift was faint, and the response varied by individual and by sex. Any changes at the metabolic level were not accompanied by clinical expressions of toxicity, as none of the subjects complained of any symptoms. The findings are especially revealing since the subjects inhaled the H_2S directly through a mouthpiece while performing moderate to strenuous exercise, thereby adding considerably to the inhaled dose of the gas compared to that which might be received under normal resting conditions. Interpretation of the clinical relevance of the findings should respect the fact that the apparent shift from aerobic metabolism to anaerobic metabolism is a routine and integral part of the body's response to increased oxygen and energy demands (i.e., physical exercise) as well as to dwindling carbohydrate reserves (i.e., fasting). It does not represent a unique response to H_2S exposure. Interpretation should also consider the fact that the subjects used in the studies were young, healthy, and generally "fit", and therefore, may not represent the general population.

Non-clinical studies:

Disturbances in acid/base balance and the mineral status of the blood, as well as shifts in the activities of various serum and tissue enzymes were reported in rabbits repeatedly exposed to 71ppm H_2S. However, confidence in the study findings and conclusions was judged to be low.

Case-control studies:

The effects of H_2S exposure on metabolic parameters were not specifically examined as part of the case-control studies reviewed

VI. OTHER CONSIDERATIONS

Several items of interest relating to the health effects associated with short-term exposures to H_2S surfaced during the course of the present review as well as during the meetings of the Expert Panel, and these items require extra consideration. The items are of scientific interest, and present rather unique challenges in terms of interpretation. They are perhaps best expressed in the form of questions. Specifically:

- What level of significance should be assigned to the deaths recorded by Weedon *et al.* (1940) and Mitchell and Yant (1925) among laboratory animals exposed to H_2S at relatively low concentrations for up to several hours?
- Why does the current review differ from the earlier review (Alberta Health, 1988) concerning the effects of short-term exposure to H_2S on the eye?
- What information can be gathered from the present review either to support or refute the increased sensitivity of certain individuals to H_2S?

Discussion around each of these items follows.

A. Mortality

Weedon *et al.* (1940) reported that 4 of 4 mice and 1 of 8 rats died following exposure to 63 ppm of H_2S for up to 16 hours. Deaths occurred within as little as 60 minutes among the exposed mice, and all mice were dead within 48 hours from start of exposure. The single rat reportedly died on test during the 16-hour exposure period. Signs and symptoms were generally mild, but consistent with discomfort and respiratory distress. Necropsy of the animals revealed signs of generalized systemic toxicity consistent with poisoning, with the lungs appearing to be most severely affected. Dose-response was evident among both species, with no deaths reported following exposure to 16 ppm of H_2S, and higher frequencies and generally shorter times to death at concentrations of 250 ppm and greater. The study received a "moderate" confidence index ranking (Study ID ● 316).

Similarly in a second older study, Mitchell and Yant (1925) reported deaths among rats exposed *"continuously"* to 100 to 140 ppm of H_2S. The deaths occurred within 18 to 48 hours following the onset of exposure. Deaths were also recorded for guinea pigs and dogs exposed to 103 ppm of H_2S, with the animals succumbing within 8 to 48 hours after the start of exposure. Canary birds were found to be especially sensitive to H_2S, with deaths recorded following exposure to 35 to 100 ppm within 4 to 18 hours after the onset of exposure. Higher concentrations resulted in a higher incidence of mortality and shorter times to death in all species tested. Signs and symptoms were consistent with irritation of the eyes, skin and/or mucous membranes, progressing to evidence of systemic toxicity in a dose-related manner. Details respecting pathology were limited. The study received a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting (Study ID ● 444).

The issue surrounding the recorded deaths concerns the considerable discrepancy between the above findings and those of the remaining studies. The mortality findings from the various studies are summarized in Appendix 7. Highlights are listed below.

- For the majority of studies, information respecting whether or not deaths occurred was missing. Although it might be reasonable to presume that had exposures caused deaths, it would have been reported, the actual results remain unknown.
- Although the focus of the present review was on exposure concentrations in the 0 to 100 ppm range, mortality data for concentrations beyond this range were included for completeness and to provide for more meaningful interpretation of the weight-of-evidence. Similarly, additional studies were included that did not satisfy the eligibility criteria *per se*, but did report mortality

findings that should bear on the interpretation of the overall data set. Both positive and negative findings emerged from this additional set of studies. The studies were excluded from detailed review since they were not peer-reviewed, did not appear as full-length publications or involved exposures that extended beyond acute or sub-acute duration. The additional set of studies should not be considered all-inclusive, but rather the studies were chosen since they used exposure concentrations within the range of interest, they were readily available, and they are often cited in the published literature.

- With only one exception, the studies received a "low" or "moderate" confidence index ranking, signaling weaknesses in experimental design, conduct or reporting. Thus, caution must be exercised in the interpretation of the findings.

- Experimental designs varied considerably. Some studies involved single exposures only, while others used repeated exposures, either intermittent or daily, over several days or weeks. Since exposure duration will directly affect the "toxic load" of H_2S received, the interpretation of the mortality data must necessarily extend beyond simple comparison of the findings across studies. No attempt was made in the present review to reach this level of interpretation.

- Apart from the findings of Hays (1972), Mitchell and Yant (1925) and Weedon *et al.* (1940), which showed deaths among various laboratory animal species following exposures to H_2S within the concentration range of interest (*i.e.*, 0 to 100 ppm), no evidence of mortality was discovered among the remaining 32 studies for the same concentration range. Confidence in the findings from the former three studies is weakened by the fact that the studies not only are "dated", but also showed weaknesses in experimental design, conduct and reporting.

- Although some exceptions occurred, the deaths observed among test animals exposed to H_2S at concentrations up to 100 ppm, typically followed continuous exposure for periods of 8 to 16 hours and beyond. In only one instance was a death reported to occur within one hour of exposure to concentrations less than 100 ppm (*i.e.*, Weedon *et al.*, 1940).

Based on the mortality data contained in Appendix 7, it would appear that the likelihood of death occurring as a result of short-term exposure to H_2S at concentrations up to 100 ppm is very remote.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

B. Eye Effects

The present review revealed very little evidence of eye irritation following short-term exposures to H_2S at concentrations up to 100 ppm. This is in sharp contrast to the earlier report (Alberta Health, 1988), wherein it was concluded that the eye *"is very susceptible to the irritant action of H_2S"*, and *"irreversible eye tissue damage can occur at 20 ppm H_2S ..."*. The discrepancy may be explained, in part, by the reliance placed on review articles in the earlier assessment. It would appear that the earlier conclusions relied heavily on statements made by Milby (1962) and Gosselin *et al.* (1976) attesting to *"irreversible eye tissue damage at concentrations $\geq$ 20 ppm for several hours' exposure"*. Neither statement represents original research. Instead, the following statement by Milby cites Yant (1930), *"exposure to concentrations above 50 ppm for a period exceeding 1 hour may produce irritation of the conjunctival and corneal tissues"*. The statement does not suggest "irreversible" eye damage, nor does it make reference to effects at 20 ppm. Moreover, the report by Yant also does not represent original research. Alberta Health (1988) also reported "*blurred vision at 0.08 ppm (Kleinfeld et al., 1964)*"; however, this exposure concentration was not stated in the cited document. Thus, it appears that unsubstantiated opinions have been propagated through several review articles, dating as far back as 1930.

As already indicated, the current review relied exclusively on original scientific findings. No reliable evidence was discovered showing eye involvement following short-term exposures to H_2S at concentrations up to 100 ppm, especially evidence of irreversible damage. Circumstantial evidence exists, but the level of confidence that can be assigned to such evidence is low. However, this is not to say that the eye is not responsive to H_2S. Rather, it simply indicates that information showing effects on the eye at concentrations up to 100 ppm is lacking. In all likelihood, given the sensitivity of the eye to irritants in general, it will respond to H_2S. The dose-responsiveness and time-course of the effect(s) at low concentrations is yet to be determined.

C. Hypersusceptibility

Although the present review was not specifically concerned with identifying, examining and/or understanding the health effects of H_2S on hypersusceptible individuals[7], information did emerge during the course of the work that could bear on this issue.

Concerns surrounding the increased susceptibility of certain individuals to the health effects of H_2S were discussed at some length in the earlier report (Alberta Health, 1988), and surfaced again during the deliberations of the Provincial Advisory Committee on Public Safety and Sour Gas (2000). Interest in this area extends back to work completed by the Illinois Institute for Environmental Quality (IIEQ, 1974), which suggested that: *"Knowledge of the toxicology of H_2S permits the identification of individuals who, because of some disease process, metabolic disorders or psychological disturbance, cannot tolerate H_2S or its by-products and therefore possess enhanced sensitivity to this gas".* Among the categories of individuals who might be affected are:

- Individuals with eye or respiratory tract problems. The suggestion is that exposure to H_2S could aggravate pre-existing conditions such as conjunctivitis, eye infections, tuberculosis, asthma, emphysema or chronic bronchitis.
- Individuals with anemia – the suggestion being that the detoxification of H_2S through oxidation via hemoglobin in the blood could be impaired in these individuals.
- Individuals with lowered resistance to bacterial or viral infections.
- Individuals with heart disease – the suggestion being that since H_2S can interfere with oxygen utilization and energy production at the cellular level, the heart muscle may be particular susceptible given its high oxygen demand and need for uninterrupted energy supply.

Findings that emerged during the present review pertaining to the issue of hypersusceptibility are discussed below:

First, the work of Jappinen *et al.* (1990) relates to the first category of individuals since testing was performed with asthmatics. Unfortunately, the results were mixed. Although the authors found that when asthmatic subjects were exposed under controlled conditions to 2 ppm of H_2S for 30 minutes *"no significant changes in respiratory function occurred"*, the changes varied considerably between individuals, with two of the ten subjects showing evidence of bronchial obstruction. The authors reported that these same subjects showed no clinical signs of respiratory discomfort or distress. Interpretation of the findings from the study should necessarily respect the fact that exposure was relatively brief (*i.e.*, 30 minutes), the exposure concentration was low (*i.e.*, 2 ppm), and that severe asthmatics were excluded from testing. On balance, the findings appear to suggest that asthmatics may be at increased risk from H_2S exposure. The degree of susceptibility will depend on the individual's condition and the exposure circumstances, but even at low concentrations of H_2S, responses might be expected. The finding is not unexpected given the etiology of the disorder and the irritant nature of the gas.

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[7] The term "hypersusceptible" was used in the context of the description provided by the Ad Hoc Committee on H_2S Toxicity (Alberta health, 1988) and referred to individuals who might be at increased risk due to pre-existing medical conditions that could be aggravated by exposure to H_2S. The term was not used to describe individuals who may be intrinsically more sensitive to chemicals in general and might respond to lower concentrations of H_2S than the general population.

Second, as outlined earlier, no firm evidence was found to confirm or refute the suggestion that the eyes will respond to short-term exposures to H_2S at concentrations in the range of interest (*i.e.*, 0 to 100 ppm). Accordingly, it is impossible to state with certainty that individuals with pre-existing eye disorders will be extra-sensitive to H_2S within this concentration range. However, first principles and "common sense" suggest that susceptibility would be heightened.

With respect to the second category of individuals (*i.e.*, anemics), no findings emerged from the present review to support the increased susceptibility of this group. The work of Bhambhani *et al.* (1994, 1996, 1997) suggested that female subjects were *less* responsive to the "metabolic" effects of short-term exposure to H_2S compared to their male counterparts despite a lower blood volume, lower hemoglobin content, and lower oxygen carrying capacity. The authors admitted that the findings were unexpected, and suggested that the inhaled dose of H_2S received by the women may have been lower than that received by the men due to a smaller inspiration volume, thereby possibly explaining the unpredicted results. In addition, the women were young, healthy and "fit", with their aerobic capacity heightened by involvement in systematic endurance training programs. As a result, it is difficult to extrapolate the findings to the general population, especially with respect to assessing whether or not the results support or refute the suggestion that anemics may be more susceptible to H_2S. Perhaps it should be pointed out that the suggestion of increased susceptibility of individuals with anemia is based strictly on theoretical grounds. In the earlier review (Alberta Health, 1988), it was concluded that the heightened susceptibility would likely only apply in cases of severe anemia.

In terms of the third category of individuals (*i.e.*, people with increased susceptibility to bacterial and viral infections), the work of Rogers and Ferin (1981) is of interest since it showed that short-term exposure of rats to a low concentration of H_2S (*i.e.*, 45 ppm) caused impairment of the pulmonary anti-bacterial defense system. These findings were supported, in turn, by the work Khan *et al.* (1991) who found that short-term exposure of rats to H_2S could reduce the viability of the pulmonary alveolar macrophages, but the response was only witnessed at a concentration of 400 ppm. No change in cell viability was evident at 50 or 200 ppm of H_2S. Although the information is limited, on balance there is some evidence to support the suggestion of heightened susceptibility to H_2S among individuals with lowered resistance to bacterial and viral infections. However, further study is needed to elucidate the mechanisms that may be involved as well as the dose-response characteristics of the changes.

With regard to the fourth category of potentially susceptible individuals (*i.e.*, people with heart disease), no reliable evidence emerged from the present review to suggest an increased risk of cardiac irregularities from short-term exposure to H_2S at low concentrations. However, as with the former categories, information is limited, and no firm evidence to contest the suggestion of increased susceptibility is available. Bhambhani *et al.* (1991,1994, 1997) did report that heart rate, systolic blood pressure and diastolic blood pressure were unaffected among volunteer subjects by brief exposure to H_2S at concentrations up to 10 ppm, prompting the authors to suggest that *"men and women with cardiovascular disease who may be exposed to H_2S due to the nature of their occupations or otherwise, may not be at an increased risk in such an environment"*. However, when interpreting the significance of these findings, it must understood that the subjects were young, healthy, and "aerobically fit", with no indication of pre-existing cardiac abnormalities. By the same token, it must be pointed out that the subjects inhaled the H_2S directly through a mouthpiece while performing moderate to strenuous exercise, thereby significantly heightening the amount of the gas received. Finally, as indicated earlier (Alberta Health, 1988) heart rate and blood pressure are imprecise indicators of cardiac toxicity.

VII. CONCLUSIONS

The conclusions that emerged from the present review are listed below. Consistent with the recommendations of the Expert Panel, the conclusions are segregated into those based on evidence from human studies and those which rely on the results from animal testing. The general basis of each concluding statement is summarized in the attached 'conclusions and commentary' summary tables (see p. 63 to 71). The tables identify the studies that featured most prominently in arriving at the conclusions. Comments bearing on the interpretation of the individual study findings are included in the tables to permit better understanding of the strengths and limitations of the conclusions. The reader is strongly encouraged to consult the full text and the detailed reviews of the individual studies (see Appendix 6) to fully appreciate the basis of the conclusions.

A. Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant adverse effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations remains unclear.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

B. Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant adverse effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting, and the toxicological significance and clinical relevance of the responses remain largely unknown.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant adverse effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects are unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms which did appear, were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al.*, 1940). In a second older study conducted by Mitchell and

Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of–evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species could not be found

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
"Young normal healthy adults can tolerate up to 10 ppm H_2S without significant adverse effects."	Bhambhani *et al.* (1996) Study ID ▲ 118 Bhambhani *et al.* (1991) Study ID ▲ 119 Bhambhani *et al.* (1994) Study ID ▲ 120 Bhambhani *et al.* (1996) Study ID ▲ 121 Bhambhani *et al.* (1997) Study ID ▲ 122	• The exposure time was limited and participants were typically young, healthy and "aerobically fit", and therefore, unlikely to be representative of the general population. • The inhaled dose was higher than that which might be received under resting conditions since the gas was administered directly via a mouthpiece while the subjects routinely engaged in moderate to strenuous exercise. • Changes were limited to alterations in pulmonary and biochemical parameters consistent with a shift from aerobic to anaerobic metabolism. Although the effects are consistent with the accepted mechanism of action of H_2S, they are not unique, but rather occur to varying degrees during normal daily activity and as part of normal diurnal rhythms. • Changes were generally mild in nature and varied by subject. Females were less responsive than males. • None of the subjects experienced any unusual signs or symptoms (*e.g.*, headache, nausea, sore throat, etc), nor any reduction in their exercise capacity.
"At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings."	Kangas and Savolainen (1986) Study ID ● 207	• No signs and symptoms were monitored. Health effects *per se* were not examined.
	Kilburn (1997) Study ID ● 216	• Decrements in neurobehavioral performance and neurophysiological function were reported among six subjects exposed to H_2S, for up to 24 hours, from a variety of point sources. The exposure concentrations to which subjects were exposed were estimated. It is likely that the exposure concentrations for some subjects were misrepresented as being in the 1 to 50 ppm range, considering that one subject was rendered unconscious and two subjects experienced olfactory paralysis at the time of exposure. • Twelve of the sixteen subjects were plaintiffs in lawsuits. Thus, subject bias my have impacted results since certain parameters were assessed based on a self-administered questionnaire. • An excessive lag period occurred between the exposure events and the testing of the subjects (*i.e.*, up to 6 years).
	Kilburn (1999) Study ID ● 217	• Neurobehavioral deficits were reported to occur in subjects exposed during the course of one week to H_2S released from a refinery explosion and fire, based on "scores" recorded through a battery of neuropsychological and neurophysiological tests. However, test scores were inconsistent for a number of the parameters monitored. The scores for most of these inconsistent responses were classified as "abnormal" by the author, with no apparent basis. • Exposure levels were estimated and inconsistently reported within the study. • An incident of this type would release numerous chemicals, apart from H_2S. These additional substances were not quantified nor was their potential impact assessed. • An excessive lag period occurred between the exposure incident and the testing of subjects (*i.e.*, more than 3 years). Consequently, other events and/or exposures occurring subsequent to the refinery explosion and fire may have contributed to the reported neurological changes. The long lag period also undermines confidence in the results, especially since the study relied, in part, on a self-administered questionnaire.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Lodgepole Blowout Inquiry Panel (1984) Study ID ● 327	• The Lodgepole well reportedly remained unignited for 26 days. During this time, the concentration of H_2S measured in the local area exceeded 10 ppm for 31 hours and 5 ppm for a period of 61hours. Concerns were raised during the inquiry over the adequacy of the monitoring equipment and the number of monitoring units employed. In fact, the Panel concluded, *"there is insufficient data to derive meaningful average hourly concentrations."* • Thirty residents within a 50km radius of the well-site complained of headache, eye irritation and upper/lower respiratory tract symptoms coincidental with the blowout. Symptoms were not verified by medical practitioners. As stated by the panel,, there was a lack *"of scientific data available for analysis"*. • It is not known to what degree the witnesses were representative of the general population within the communities surrounding the well-site. Subject bias was possible. • The role of other chemical agents or other possible causative factors in inducing the observed symptoms was not explored.
	Alberta Social Services and Community Health (1983) Study ID ● 424	• During the Lodgepole blowout, the well was unlit for a total of 28 days, during which time, local monitoring stations assessed peaks levels to be ≤ 5.5 ppm. However, the actual concentration to which people were exposed is unknown, since the precise location of monitoring equipment with respect to the peoples' homes was not stated. • Complaints filed with Alberta Environment during this period included general malaise, headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, etc. All symptoms were self-reported and not verified by medical practitioners. Symptom frequency in an unexposed population was not included in the analysis. • The percent of residents adversely affected by the release was not reported. Thus, no conclusions with respect to the universality of the signs and symptoms can be stated. • As a highly publicized event, subject bias likely skewed results of the study.
	Mitchell and Yant (1925) Study ID ● 444	• Male subjects exposed to 100-150 ppm of H_2S reportedly experienced coughing, irritation of the eyes and loss of sense of smell within 2 to 15 minutes. Symptoms progressed in severity with continued exposure, with salivation and mucous discharge and a sharp pain in the eyes reportedly after 1 to 4 hours of exposure. • Study was described by the authors as being "preliminary". • Male subjects only were used. • Details concerning exposure conditions, exposure chamber, and gas delivery system were generally lacking. • Details concerning subjects (*e.g.*, age, health status) were completely lacking.
"…short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance…Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations remains unclear.	Jappinen *et al* (1990) Study ID ◆ 202	• The reported changes in airway resistance (*i.e.*, 26.3% increase on average) and airway conductance (*i.e.*, 8.4% decrease on average) were not statistically significant. • Some subjects were non-responsive or showed decreased airway resistance and/or increased airway conductance. • The response appears to be very much subject-dependent and possibly influenced by the severity of the asthmatic condition. In 2 of the 10 exposed asthmatics marked changes in airway conductance and resistance indicative of bronchial obstruction were reported, however, no signs or symptoms consistent with respiratory discomfort or distress were observed. • Subjects had a history of mild to moderate asthma. Individuals with severe asthma were excluded from the study. • The authors noted that the results were preliminary and required further study. They concluded that the exposure caused *"no specific changes in respiratory function"*. and *"although the*

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
		airway resistance increased somewhat after exposure, it was not reflected in the subjects' clinical condition". • The exposure duration was brief and the exposure concentration relatively low. The authors concluded that: *"it is not possible to predict what kind of symptoms asthmatic subjects could have at higher concentrations".*
"No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified."	No studies complied with the eligibility criteria.	• Not applicable.

[1] The reader is strongly encouraged to consult the individual study reviews found in Appendix 6 to obtain a full understanding of the various factors that should be considered in the interpretation of the findings and conclusions from the cited studies.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
"short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant adverse effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting, and the toxicological significance and clinical relevance of the responses remains largely unknown."	Dorman, *et al* (2002) Study ID ◆ 151	• The only change observed following nose-only exposure of male rats to 30 ppm H_2S was inhibition of cytochrome oxidase in the lung, nasal olfactory epithelium and nasal respiratory epithelium. In the liver, an opposite response was observed, the activity of cytochrome oxidase increased in response to exposure to concentrations at low as10 ppm. In the absence of conventional measures of toxicity, the clinical significance of the observed changes is unknown.
	Haider *et al* (1980) Study ID ● 179	• Whole-body exposure of male guinea pigs to 20 ppm H_2S for 1h/d for 11 days reportedly caused regional-specific reductions in total lipids and phospholipids in brain tissue and increased lipid peroxidation. The changes were not consistent across all regions of the brain, and varied according to lipid type, rendering the interpretation of the significance of the findings difficult. • Evidence of eye irritation was reported, but was not confirmed by *in situ* ophthalmoscopic examination.
	Lopez *et al* (1987) Study ID ◆ 231	• A transient increase in the absolute number of epithelial cells was detected in the nasal lavage fluid of male rats exposed to 10 ppm H_2S in a whole-body chamber. The authors cautioned that the toxicological significance of the response should not be overestimated *"since dramatic change can rapidly be restored due to remarkable reparative capacity of respiratory epithelium."*
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 25 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.
	Weedon *et al.*(1940) Study ID ◆ 316	• Mild dose-dependent signs of intoxication (*e.g.*, transient restlessness) were observed in rats exposed to 16 ppm H_2S. Necropsy findings at this concentration were *uniformly non-remarkable*. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation and the associated uncertainty surrounding the actual exposure concentrations that were tested.
"Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations…continued to support an absence of significant adverse effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects are unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms which did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed."	Khan *et al* (1990) Study ID ◆ 210	• Effects of whole body exposure of male rats to 50 ppm H_2S for 4 hours were limited to a reduction in cytochrome *c* oxidase activity. However, the level of inhibition detected post-exposure (*i.e.*, 15%) was comparable to the variability observed among control rats (*i.e.*, 18%). Despite, the enzyme inhibition, no signs and symptoms of toxicity were reported.
	Rogers and Ferin (1981) Study ID ◆ 269	• Exposure to 45 ppm H_2S over a period of 2 hours resulted in no significant changes in the pulmonary antibacterial defense capacity of exposed male rats; however, following 4 and 6 hours of exposure bacteria inactivation was significantly reduced. Time-responsiveness of the effect was demonstrated through the use of graded exposure times, but due to the use of a single exposure concentration the dose-responsiveness of the change could not be determined. Further, the manner by which control rats were handled was not entirely clear, thereby rendering interpretation of the findings difficult.
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 50 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Lopez *et al.* (1986) Study ID ◆ 466	• Exposure of male rats to 40 ppm H_2S for 6-hours resulted in initial weight loss and clinical signs of intoxication (*e.g.*, agitation, lacrimation, and moderate respiratory distress). Pathological examination post-exposure detected focal necrosis of the nasal epithelium and mild pulmonary oedema. However, responses were transient and generally resolved within hours post-exposure (*e.g.*, repair of the focal necrosis of the nasal epithelium was obvious at 42 hours post-exposure). In addition, the observed effects of H_2S on the nasal epithelium might be expected to be more severe in the rat compared to humans since rats are obligate nasal breathers.
	Mitchell and Yant (1925) Study ID ● 444	• Death among canary birds following exposure to 35 – 65ppm for 8 to 18 hours. Canaries were described by authors as being extremely susceptible to poisonous gases. • Signs and symptoms among rats and guinea pigs exposed to 35-65ppm for as little as 1 to 4 hours. Signs and symptoms consisted of eye irritation and rough hair coat, which progressed in severity with continued exposure up to 100 hours. No deaths. • Information respected exposure conditions, exposure chamber and gas delivery system were generally lacking. • Study is very "dated".
"Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations…were very conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one study. Serious adverse effects, including death, were absent from all of the remaining studies. The reason(s) for the discrepancy in findings is not known. However, the weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely."	Dorman *et al.* (2002) Study ID ◆ 151	• Transient shifts in tissue sulfide content, cytochrome oxidase activity and/or the concentrations of sulfide metabolites were observed in male rats exposed to 80 ppm H_2S via a nose-only inhalation apparatus. In the absence of conventional measures of toxicity, the clinical significance of the observed changes is unclear.
	Elovaara *et al.* (1978) Study ID ● 155	• Whole body exposure of female mice to 100 ppm H_2S for 2 hours reportedly resulted in alterations in protein metabolism in the brain, although the protein content of both the brain and myelin was unaffected. The effect was inconsistent, varying with the duration of exposure. The increase in leucine incorporation detected during the first 24 hours of exposure was followed by a decrease relative to controls. This shift in response renders interpretation of the findings difficult. • Apart from transient agitation during the first 20 minutes of exposure, the mice remained clinically unaffected. Thus, the changes in protein metabolism were not accompanied by observable signs or symptoms of intoxication. No gross or histopathological examination of the brain or myelin tissues was performed to determine if the changes had macroscopic or microscopic correlates.
	Kosmider *et al.* (1967) Study ID ● 223	• Acute and subacute exposure of rabbits to 71 ppm H_2S reportedly caused electrocardiographic and histochemical changes in the heart muscle. Interpretation of the significance of the studies findings was limited by reporting deficiencies (*e.g.*, lack of control data). • Animals exposed for a single 1.5 hour exposure period lost consciousness, whereas those exposed repeatedly for 30 min/day for 5 days exhibited no signs or symptoms. In studies conducted by Kosmider in 1967 (Study ID ● 694) and 1971 (Study ID ● 595), rabbits exposed for 1.5 hours and 1 hour, respectively, were not reported to be rendered unconscious during exposure.
	Lopez *et al.* (1988) Study ID ◆ 232	• Changes to the morphological architecture of the lungs of male rats exposed to 83 ppm H_2S for four hours were relatively minor (*e.g.*, perivascular edema of pulmonary vessels and distention of perivascular space) and resolved quickly following cessation of exposure. These changes were not accompanied by observable signs or symptoms of intoxication.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Savolainen *et al.* (1980) Study ID ● 280	• Mice exposed to 100 ppm of H_2S for 2 hours at four-day intervals for up to 16 days reportedly showed cumulative changes in a number of metabolic and enzymatic cerebral parameters (*e.g.*, cytochrome oxidase activity, RNA synthesis, superoxide dismutase activity, glutathione levels, etc.). However, the changes rarely achieved statistical significance and generally varied less than 10% from control values. In addition, the nature of the change (*i.e.*, increase vs. decrease) for some parameters was not consistent across the entire treatment schedule. Thus, a treatment-related effect was not obvious. • Apart from some initial "excitement" at the initiation of each exposure period, metabolic and enzymatic changes were not accompanied by any observable signs or symptoms of intoxication.
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 75 ppm and 100 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.
	Struve *et al.* (2001) Study ID ◆ 296	• The motor activity of male rats receiving whole-body exposure to 80 ppm H_2S for 3h/d for five consecutive days was unchanged, whereas animals that received nose-only exposures showed reduced activity. However, the change was significant at only 2 of 10 testing intervals. Given the inconsistency of the response and the lack of progression of the change over the course of treatment, combined with the lack of response for all other measures of neurobehavioral function, the change in spontaneous motor activity was judged to be equivocal.
	Weedon *et al.* (1940) Study ID ◆ 316	• Rats and mice exposed to 63 ppm H_2S for up to 16 hours exhibited frank evidence of intoxication, especially among test mice, with deaths recorded as early as within one hour of exposure. All of the mice (4 of 4) and one of eight rats were dead within 40 hours. Considerable discrepancy exists between this finding and those reported by more recent studies. Specifically, whereas Weldon *et al.* reported deaths within 60 minutes, both Elovaara *et al*, 1978 (Study ID ● 155) and Savolainen *et al*, 1980 (Study ID ● 280) reported no deaths among mice following exposure to 100 ppm H_2S for 2 hours. Thus, it would appear from the available information that the high incidence of deaths reported by Weedon *et al.* is unusual. The results may reflect the age of the study since the exposure equipment and gas monitoring systems were necessarily "crude" by present-day standards. Some uncertainty exists surrounding the actual exposure concentrations to which the animals on test were exposed.
	Nicholson *et al.* (1998) Study ID ◆ 398	• Whole body exposure of male rats to 100 ppm H_2S for 3h/d for 5 days reportedly increased levels of L-glutamate in the hippocampus. The dose-responsiveness of this change could not be assessed since only a single exposure concentration was employed. The authors' claim that elevated L-glutamate levels may, due to their role as a neurotransmitter, contribute to cerebral neurotoxicity was not substantiated since no symptomatic or pathological correlates of the observed changes were reported owing to limitations in study design.
	Mitchell and Yant (1925) Study ID ● 444	• Signs and symptoms consistent with systemic toxicity (e.g., dizziness, laboured respiration, and unconsciousness) among canary birds following exposure to 100ppm for 1 to 8 hours. Deaths within 4 to 8 hours. • Deaths among guinea pigs (1 of 2), dogs (1 of 2) and rats (?) following exposure to 100-140ppm for at least 8 hours.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Hulbert *et al.* (1989) Study ID ● 460	• One-hour exposure to 100 ppm H_2S reportedly produced a transient decrease in airway resistance and an increase in dynamic compliance in 50% of the guinea pigs under test. The response is contrary to that which might be expected given the irritant properties of H_2S and the commonly reported broncho-obstruction following exposure. • The mode of administration of H_2S involved intra-tracheal treatment of the animal under restraint, aided by mechanical ventilation. Consequently, the gas by-passed the "scrubbing" action provided by the mucosal epithelium lining the upper respiratory passages and therefore, the findings likely over-state the changes that might occur in the pulmonary tissue of a exposed human. • Microscopic examination of pulmonary tissue exposed to 100 ppm H_2S revealed no apparent damage.
	Kosmider *et al.* (1971) Study ID ● 595	• Rabbits exposed for 1h/d for 14 days reportedly exhibited changes in serum and/or tissue protein, mineral and enzyme levels. Clinical relevance was difficult to establish since the study did not outline the range of shifts typical of normal homeostatic mechanisms. According to the authors, some changes were indicative of liver and kidney damage; however, no histopathological evaluations were conducted to verify this conclusion. • Conventional measures of toxicity were largely lacking, but rapid respiration, elevated heart rate and initial excitement were observed at the time of exposure. • Eyes reportedly developed "congestion" of the conjunctiva, but the nature of the congestion was poorly described and was not investigated by *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination.
	Kosmider *et al.* (1967) Study ID ● 694	• Exposure of rabbits to 71 ppm H_2S for 1.5 hours reportedly resulted in a decrease in both serum and brain alkaline phosphatase activity. The clinical and toxicological significance of these changes are unknown. • No signs or symptoms were reported.
"No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen. Comparable studies using other animal species could not be found."	Hannah and Roth (1991) Study ID ● 11	• Offspring of pregnant female rats exposed to 20 and 50 ppm H_2S from day 5 post-coitus until day 21 post-natal were reported to exhibit abnormal development at the "growth front" of the cerebellar Purkinje cells. Considering specie specific gestation periods, the equivalent exposure time for humans would extend over several months. This coupled with the lack of dose-responsiveness and the minimal degree of change observed renders interpretation of the significance of the findings difficult. • The absence of clinical data precludes any consideration as to whether the reported changes may result in behavioral abnormalities or if the architectural alterations represent direct H_2S toxicity or secondary responses to maternal stress.
	Kosmider *et al.* (1971) Study ID ● 595	• Rabbits exposed for 1h/d for 14 days reportedly exhibited changes in serum and/or tissue protein, mineral and enzyme levels. Clinical relevance was difficult to establish since the study did not outline the range of shifts typical of normal homeostatic mechanisms. According to the authors, some changes were indicative of liver and kidney damage; however, no histopathological evaluations were conducted to verify this conclusion. • Conventional measures of toxicity were largely lacking, but rapid respiration, elevated heart rate and initial excitement were observed at the time of exposure. • Eyes reportedly developed "congestion" of the conjunctiva, but the nature of the congestion was poorly described and was not investigated by *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Kosmider *et al.* (1967) Study ID ● 694	• Exposure of rabbits to 71 ppm H_2S for 1.5 hours reportedly resulted in a decrease in both serum and brain alkaline phosphatase activity. The clinical and toxicological significance of these changes are unknown. • No signs or symptoms were reported.
"No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen. Comparable studies using other animal species could not be found."	Hannah and Roth (1991) Study ID ● 11	• Offspring of pregnant female rats exposed to 20 and 50 ppm H_2S from day 5 post-coitus until day 21 post-natal were reported to exhibit abnormal development at the "growth front" of the cerebellar Purkinje cells. Considering specie specific gestation periods, the equivalent exposure time for humans would extend over several months. This coupled with the lack of dose-responsiveness and the minimal degree of change observed renders interpretation of the significance of the findings difficult. • The absence of clinical data precludes any consideration as to whether the reported changes may result in behavioral abnormalities or if the architectural alterations represent direct H_2S toxicity or secondary responses to maternal stress.
	Hayden *et al.* (1990) Study ID ◆ [illegible]	• Exposure of gravid rat dams to 20, 50, or 75 ppm H_2S from day 6 of gestation to day 21 post-partum reportedly caused dystocia. However, the reported dystocia was based on the observation of a limited number of animals (*i.e.*, about ⅓ of the number recommended by guidelines for developmental toxicity studies). Moreover, the actual incidence of the dystocia was not reported. Large standard deviations of mean parturition times indicate a high degree of inter-animal variability. Parturition times also varied across trials, as evidenced by differences in the times reported for each of the control groups. Consequently, it is very difficult to assign significance to the findings. • A transient depression in feed intake was noted in treated dams during the first few days following initiation of treatment; however, normal feed intake was restored within one week and the body weights of treated dams were unaffected. • A 6% increase in maternal liver cholesterol was noted following exposure to 75 ppm. However, this did not appear to be a dose-responsive change as exposure to 20 ppm and 50 ppm H_2S decreased maternal cholesterol relative to controls. • The reported changes in offspring development (*e.g.*, altered pinna detachment and hair development) were not dose-responsive and did not uniformly achieve statistical significance. • All remaining maternal parameters and pup parameters monitored were unaffected (*e.g.*, organ weights, liver/brain protein and DNA content, brain cholesterol levels, etc.). Similarly, routine reproductive parameters were normal.
	Dorman *et al.* (2000) Study ID ▲ 149 CIIT (1999) Study ID ▲ 594	• The response of male and female rats exposed to 0, 10, 30, or 80 ppm H_2S for 6h/d during the course of a two-week pre-breeding period, a two-week mating period, the gestation period and a three-week postnatal period was limited to a transient reduction in body weight and feed consumption among the high-dose F_0 males, a typical response to a chemical stressor in laboratory rodents. • Any differences in reproductive, developmental or pathological parameters were generally isolated, minor in nature, non-dose-related and/or of unknown biological significance. Although, malformations were only observed in newborn pups from dams exposed to H_2S, careful review of the incidence of the malformations revealed no evidence that the structural defects were related to treatment. The majority of the pups were from a single litter and the most common structural defects were described as "skin and dermis detachment", which was likely of genetic origin. • The authors concluded that the findings suggest that H_2S is neither a reproductive toxin nor teratogen.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Hannah *et al.* (1989) Study ID ● 180	• Exposure of pregnant rats to 75 ppm H_2S for 7h/day from day 5 post-coitus until day 21 resulted in alterations in levels of selected amino acids in regions of the brain in the offspring. However, variations in regional and temporal responses, and the absence of symptomatic data make the interpretation of the significance of the changes difficult. Further, the equivalent exposure time in humans would extend over several months, an exposure period which would be unlikely to occur at this exposure concentration.
	Saillenfait *et al.* (1989) Study ID ◆ 778	• Exposure of pregnant rats to 50, 100, and 150 ppm H_2S reportedly resulted in a dose-related change in mean implantation sites per litter and changes in both maternal and fetal body weights. However, the changes were confined to the higher exposure groups and replicate experiments yielded different degrees of change in certain parameters.
	Skrajny *et al* (1992) Study ID ◆ 290	• *In utero* and post-natal exposure of rats to 20 ppm and 75 ppm H_2S for 7h/d from day 5 post-coitus to day 21 post-partum reportedly resulted in changes in the levels of serotonin and norepinephrine in the developing cerebellum and/or frontal cortex. However, a lack of dose-responsiveness, inconsistency in the nature of responses at different sampling times and the absence of observational, histopathological and histochemical data renders interpretation of the findings very difficult. The changes were of unknown biological significance and clinical relevance.
	Hannah *et al.* (1990) Study ID ● 402	• Exposure of pregnant rats to 50 ppm H_2S for 7h/d from day 6 post-coitus until day 21 postnatal was associated with an average 30% increase in maternal blood taurine levels relative to corresponding control values. Interpretation of the significance of the findings with respect to animal health is hampered by the absence of observational data. The clinical relevance of the finding is unclear.
	Roth and Hannah (1989) Study ID ● 630	• *In utero* and post-natal exposure of rat pups to 75 ppm H_2S for 7h/d from day post-coitus to day 21 post-partum reportedly produced morphological changes in the cerebellum (*i.e.*, increased number of Purkinje cells). However, the lack of routine measures of toxicity (*e.g.*, observational data) prevents conclusions pertaining to the overall impact of the observed increase in Purkinje cell density on the functional integrity of the CNS. Further, the equivalent exposure time in humans would extend over several months, an exposure period which would be unlikely to occur at this exposure concentration.

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[1] The reader is strongly encouraged to consult the individual study reviews found in Appendix 6 to obtain a full understanding of the various factors that should be considered in the interpretation of the findings and conclusions from the cited studies.

VIII. SUMMARY REMARKS

The Terms of Reference for the present work called for a review of the currently available health effects information surrounding short-term exposures to low concentrations of hydrogen sulphide. The review was prompted, in part, by the recommendations of the Provincial Advisory Committee on Public Safety and Sour Gas, which specifically encouraged development of a *"revised comprehensive health effects table"* for H_2S to replace the version first prepared in 1988 on the basis of the conclusions reached by the Ad Hoc Committee on H_2S Toxicity following a review of the scientific literature available at that time (Alberta Health, 1988). The earlier review assessed the scientific evidence for acute, sub-chronic and chronic effects from exposure to H_2S at concentrations up to 20 ppm. It concluded, in part, that:

1. *H_2S by itself is a broad-spectrum toxicant that can elicit numerous psychological and biological responses in the 0 to 20 ppm range with which this review was concerned. As with any chemical, all organ systems respond variably to different levels of H_2S with no given level affecting all systems equally at the same time or rate.*
2. *In general, single or repeated exposures of "normal, healthy adults" to H_2S in the 0 to 5 ppm range have not resulted in clinically-detectable irreversible biological or psychological effects. Whether this is true for hypersusceptible individuals is not currently known.*
3. *Although reversible adverse effects may result in temporary discomfort or changes in organ function, they have not been found to have a lasting impact to date.*

These conclusions helped to shape the 1988 version of the "health effects table".

In arriving at the conclusions from the current review of the health effects information on H_2S (see Chapter VII: Conclusions), several items surfaced that had a direct bearing on the meaning and level of significance that could be assigned to the collective findings from the various studies that were reviewed. An understanding of these items is critical since they speak directly to the nature of the conclusions that were developed and the limitations thereof. They also account for differences in the conclusions reached in the present review from those reached in 1988. The items are captured in the following questions:

- What major uncertainties exist in the health effects information surrounding short-term exposures to H_2S, and how do these uncertainties affect the conclusions concerning potential effects on humans?
- What limitations surround the current health effects database on short-term exposures to H_2S, and how does these limitations affect the strength of the conclusions?
- How does the present review differ from that completed in 1988, and how do these differences affect the current set of conclusions?

Discussion around each of these items follows.

A. Uncertainties in Information

Uncertainty always surrounds scientific research and the conclusions that can be drawn. The uncertainty takes many forms, ranging from that resulting from data gaps or missing information to that associated with potential errors in sampling or measurement. Such uncertainty is not unique to the health effects information on short-term exposures to H_2S. Rather, it applies to virtually all forms of scientific investigation. In the case of toxicological research aimed at understanding health effects, an additional layer of uncertainty exists in the form of intra-species (*i.e.*, within species) and inter-species (*i.e.* between species) variability that can affect the response(s) to a chemical and any associated health outcomes. This variability essentially precludes direct extrapolation of findings from animal testing to the human condition without careful consideration of species differences. In addition, it can preclude extrapolation of results from clinical or case-control studies to the general population in light of individual differences in response.

The uncertainty associated with inter-species variability can be addressed through different means, ranging from the systematic investigation of differences at the physiological, functional, structural and/or metabolic levels between species to better understand how the findings might apply to the human condition, to the largely arbitrary use of "safety factors" which assumes that humans are more sensitive than test animals. Similar approaches can also be applied to account for intra-species differences.

Notwithstanding the above, no attempt was made in the present review to extrapolate the findings from the animal studies to the human condition. Rather, the information was simply segregated according to study type, with the clinical and non-clinical data clearly differentiated. No attempt was made to adjust the findings to account for differences between species in factors such as pulmonary dynamics and pharmacokinetics that would act to determine the "toxic load" of H_2S received in each of the studies reviewed. Similarly, no attempt was made to extrapolate the findings from the clinical and case-control studies to the general population. Such exercises were considered to be beyond the scope of the current literature review. This position was accepted by the Expert Panel, with the proviso that the clinical and non-clinical information be clearly distinguished.

In light of the above, the conclusions arising from the present review have been separated into those based on evidence from human studies and those based on results from animal testing. Caution must be exercised in the interpretation and use of the latter conclusions since they should not be directly extrapolated to the human condition without further analysis.

B. Limitations in Information

The present review revealed some rather serious limitations in the scope and quality of information respecting the health effects of short-term exposures to H_2S. These limitations affected the strength of the conclusions that were drawn. In some respects, the limitations remained the same as those identified by the Ad Hoc Committee on H_2S Toxicity in 1988. For example, the earlier review made frequent reference to the need for *"further research"* owing to the *"lack of good scientific data"* and the *"little attention"* afforded to the study of the effects of H_2S on certain organ systems. Although a significant body of new information has emerged since 1988, particularly with respect to reproductive and developmental toxicity, data on many systems remain lacking. Moreover, a considerable amount of the earlier and newer work is of limited usefulness because of weaknesses in experimental design, conduct and/or reporting, which preclude critical interpretation of the biological significance and clinical relevance of the findings. The level of confidence that could be assigned to the findings and conclusions from this work was judged to be very limited. Examples of the limitations were provided earlier, but they are repeated here since they are especially telling and must be considered in the overall interpretation and use of the health effects information.

- Only *one* of the studies reviewed was performed under Good Laboratory Practice (GLP) conditions. No reference to GLP was discovered in any of the remaining studies.
- Close to 50 percent of the studies received a "low" confidence index ranking, indicating serious weaknesses in study design, execution and/or reporting when measured against recommended testing guidelines developed by leading scientific and regulatory authorities. Only 15 percent of the studies achieved a "high" confidence index ranking.
- Significant departures from even basic protocol requirements were common. Close

to 50 percent of the controlled studies used only a single exposure concentration, thereby precluding assessment of the dose-responsiveness of any observed changes and hindering any conclusions as to whether or not responses were truly treatment-related. Forty percent of the studies involved use of a single sex, thereby limiting the extrapolation of the results to the general population.

- Close to 80 percent of the non-clinical studies followed unconventional designs, hindering both the interpretation of the results and the comparison of findings across studies as a means to assess the reproducibility of any observed outcomes.
- The bulk of information respecting the health effects of short-term exposures to H_2S was derived from animal testing. Human data were very limited, further highlighting the uncertainty surrounding the extrapolation of the health effects information to the general human population.
- For many organ systems, reliable information on effects following short-term exposure to H_2S is almost completely lacking. Very little information was found with respect to effects on the skin, liver, kidney, gastrointestinal tract, hematopoietic and immunological systems. However, effects on the nervous system (*i.e.*, the primary systemic target) and respiratory system (*i.e.*, the portal of entry for inhaled H_2S) were well documented.

Based on the above shortcomings, the need for further research on the health effects of H_2S still remains. The requirement that future research follow recommended testing guidelines should be strongly encouraged. Such work will likely allow for the conclusions respecting the health effects of short-term exposure to H_2S at concentrations up to 100 ppm to be strengthened and broadened. In the meantime, the conclusions reached in the present review are judged to accurately reflect the currently available scientific information. Although circumstantial evidence exists that possibly contests certain of the conclusions, the information should be viewed as questionable and should not be relied upon as part of a scientific review.

C. Comparison with 1988 Review

The present review bears some resemblance to the earlier review completed by the Ad Hoc Committee on H_2S Toxicity, which ultimately led to the development of the 1988 "health effects table". Both the current review and the earlier work focused on the potential health effects associated with low-level exposures to H_2S, and in each case, "low level" was defined as concentrations up to 100 ppm. In addition, both reviews involved an extensive review of the scientific literature on the health effects of H_2S. Third, both reviews "weighed" the quality of the scientific information, albeit to different degrees. Further, both reviews relied on technical expertise as well as professional judgement in assessing the information. Finally, both reviews were performed under the auspices of an expert committee or panel, with members drawn from various stakeholder groups.

Despite these similarities, important differences in the approach used in each review become evident on closer examination. Specifically, the present review:

- Captured health effects information found in the scientific literature published up to 2002. (The earlier review was necessarily limited to publications appearing before 1988).
- Focused only on the assessment of health effects following acute or sub-acute exposures, extending over a few hours or a few days. (The former review included sub-chronic and chronic exposures, lasting several months or several years).
- Relied only on information found in "primary" papers, in which the original research work was described. (The earlier review placed more reliance on abstracts and review articles).
- Used a pre-defined set of objective "quality" criteria to gauge the technical strengths and

weaknesses of each scientific paper, and to assess the level of confidence that could be assigned to the study findings and conclusions. (The former review simply used professional judgement to "weigh" the quality of the information according to its source).

- Focused on the responses that might follow short-term exposure to H_2S at low concentrations on "healthy" individuals, with no specific prediction of the potential effects of H_2S on "hypersusceptible" people. Nevertheless, any specific information on "hypersusceptible" people should have been captured by this review. (The earlier review sought to clarify the effects of low levels of H_2S on sensitive individuals).
- Clearly distinguished effects on the basis of study type, with the evidence from human studies deliberately segregated from the results of animal testing. (In the former review, the information from clinical and non-clinical studies was merged and conclusions were common to both study types).

These differences in approach are important since they have a direct bearing on the interpretation of the health effects information presented in each review, as well as on the conclusions drawn. Accordingly, side-by-side comparison of the two sets of conclusions is not easily performed. Any comparison must necessarily carefully weigh the differences in approach used as well as the source and basis of the statements.

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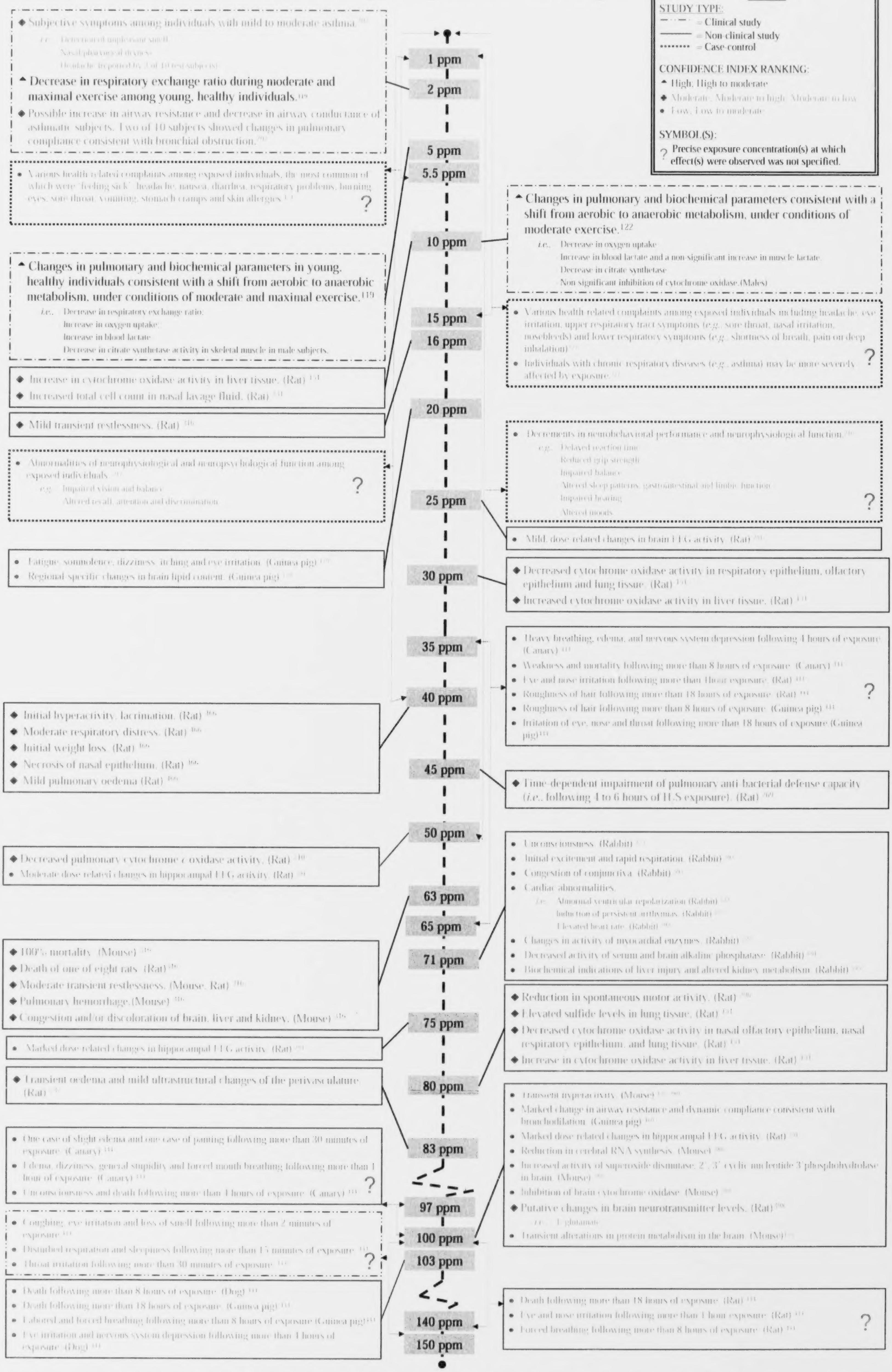
Figure 1: Health effects associated with short-term low level exposure to H_2S.

Summary of "positive" findings.*



KEY

STUDY TYPE:

- — · · — = Clinical study
- ——— = Non-clinical study
- ········ = Case-control

CONFIDENCE INDEX RANKING:

- ▲ High; High to moderate
- ◆ Moderate; Moderate to high; Moderate to low
- ● Low; Low to moderate

SYMBOL(S):

? Precise exposure concentration(s) at which effect(s) were observed was not specified.

- ◆ Subjective symptoms among individuals with mild to moderate asthma.
  - *i.e.*, Detection of unpleasant smell
  - Nasal/pharyngeal dryness
  - Headache (reported by 3 of 10 test subjects)
- ▲ Decrease in respiratory exchange ratio during moderate and maximal exercise among young, healthy individuals.[119]
- ◆ Possible increase in airway resistance and decrease in airway conductance of asthmatic subjects. Two of 10 subjects showed changes in pulmonary compliance consistent with bronchial obstruction.

- ● Various health related complaints among exposed individuals, the most common of which were "feeling sick", headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, vomiting, stomach cramps and skin allergies. ?

- ▲ Changes in pulmonary and biochemical parameters in young, healthy individuals consistent with a shift from aerobic to anaerobic metabolism, under conditions of moderate and maximal exercise.[119]
  - *i.e.*, Decrease in respiratory exchange ratio;
  - Increase in oxygen uptake;
  - Increase in blood lactate
  - Decrease in citrate synthetase activity in skeletal muscle in male subjects.

- ◆ Increase in cytochrome oxidase activity in liver tissue. (Rat)
- ◆ Increased total cell count in nasal lavage fluid. (Rat)

- ◆ Mild transient restlessness. (Rat)

- ● Abnormalities of neurophysiological and neuropsychological function among exposed individuals. ?
  - *e.g.*, Impaired vision and balance
  - Altered recall, attention and discrimination

- ● Fatigue, somnolence, dizziness, itching and eye irritation. (Guinea pig)
- ● Regional specific changes in brain lipid content. (Guinea pig)

- ◆ Initial hyperactivity, lacrimation. (Rat)[166]
- ◆ Moderate respiratory distress. (Rat)[166]
- ◆ Initial weight loss. (Rat)[166]
- ◆ Necrosis of nasal epithelium. (Rat)[166]
- ◆ Mild pulmonary oedema (Rat)[166]

- ◆ Decreased pulmonary cytochrome *c* oxidase activity. (Rat)
- ● Moderate dose related changes in hippocampal EEG activity. (Rat)

- ◆ 100% mortality. (Mouse)[316]
- ◆ Death of one of eight rats. (Rat)[316]
- ◆ Moderate transient restlessness. (Mouse, Rat)[316]
- ◆ Pulmonary hemorrhage. (Mouse)[316]
- ◆ Congestion and/or discoloration of brain, liver and kidney. (Mouse)[316]

- ● Marked dose related changes in hippocampal EEG activity. (Rat)

- ◆ Transient oedema and mild ultrastructural changes of the perivasculature. (Rat)

- ● One case of slight edema and one case of panting following more than 30 minutes of exposure. (Canary)[111]
- ● Edema, dizziness, general stupidity and forced mouth breathing following more than 1 hour of exposure. (Canary)[111] ?
- ● Unconsciousness and death following more than 4 hours of exposure. (Canary)[111]

- ● Coughing, eye irritation and loss of smell following more than 2 minutes of exposure.[111]
- ● Disturbed respiration and sleepiness following more than 15 minutes of exposure.[111] ?
- ● Throat irritation following more than 30 minutes of exposure.[111]

- ● Death following more than 8 hours of exposure. (Dog)[111]
- ● Death following more than 18 hours of exposure. (Guinea pig)[111]
- ● Labored and forced breathing following more than 8 hours of exposure (Guinea pig)[111]
- ● Eye irritation and nervous system depression following more than 4 hours of exposure. (Dog)[111]

- ▲ Changes in pulmonary and biochemical parameters consistent with a shift from aerobic to anaerobic metabolism, under conditions of moderate exercise.[122]
  - *i.e.*, Decrease in oxygen uptake
  - Increase in blood lactate and a non-significant increase in muscle lactate.
  - Decrease in citrate synthetase
  - Non significant inhibition of cytochrome oxidase. (Males)

- ● Various health related complaints among exposed individuals including headache, eye irritation, upper respiratory tract symptoms (*e.g.*, sore throat, nasal irritation, nosebleeds) and lower respiratory symptoms (*e.g.*, shortness of breath, pain on deep inhalation) ?
- ● Individuals with chronic respiratory diseases (*e.g.*, asthma) may be more severely affected by exposure.

- ● Decrements in neurobehavioral performance and neurophysiological function. ?
  - *e.g.*, Delayed reaction time
  - Reduced grip strength
  - Impaired balance
  - Altered sleep patterns, gastrointestinal and limbic function
  - Impaired hearing
  - Altered moods

- ● Mild, dose related changes in brain EEG activity. (Rat)

- ◆ Decreased cytochrome oxidase activity in respiratory epithelium, olfactory epithelium and lung tissue. (Rat)
- ◆ Increased cytochrome oxidase activity in liver tissue. (Rat)

- ● Heavy breathing, edema, and nervous system depression following 4 hours of exposure (Canary)[111]
- ● Weakness and mortality following more than 8 hours of exposure. (Canary)[111]
- ● Eye and nose irritation following more than 1 hour exposure. (Rat)[111] ?
- ● Roughness of hair following more than 18 hours of exposure. (Rat)[111]
- ● Roughness of hair following more than 8 hours of exposure. (Guinea pig)[111]
- ● Irritation of eye, nose and throat following more than 18 hours of exposure (Guinea pig)[111]

- ◆ Time-dependent impairment of pulmonary anti-bacterial defense capacity (*i.e.*, following 4 to 6 hours of H_2S exposure). (Rat)

- ● Unconsciousness. (Rabbit)
- ● Initial excitement and rapid respiration. (Rabbit)
- ● Congestion of conjunctiva. (Rabbit)
- ● Cardiac abnormalities.
  - *i.e.*, Abnormal ventricular repolarization (Rabbit)
  - Induction of persistent arrhythmias. (Rabbit)
  - Elevated heart rate. (Rabbit)
- ● Changes in activity of myocardial enzymes. (Rabbit)
- ● Decreased activity of serum and brain alkaline phosphatase. (Rabbit)
- ● Biochemical indications of liver injury and altered kidney metabolism. (Rabbit)

- ◆ Reduction in spontaneous motor activity. (Rat)
- ◆ Elevated sulfide levels in lung tissue. (Rat)
- ◆ Decreased cytochrome oxidase activity in nasal olfactory epithelium, nasal respiratory epithelium, and lung tissue. (Rat)
- ◆ Increase in cytochrome oxidase activity in liver tissue. (Rat)

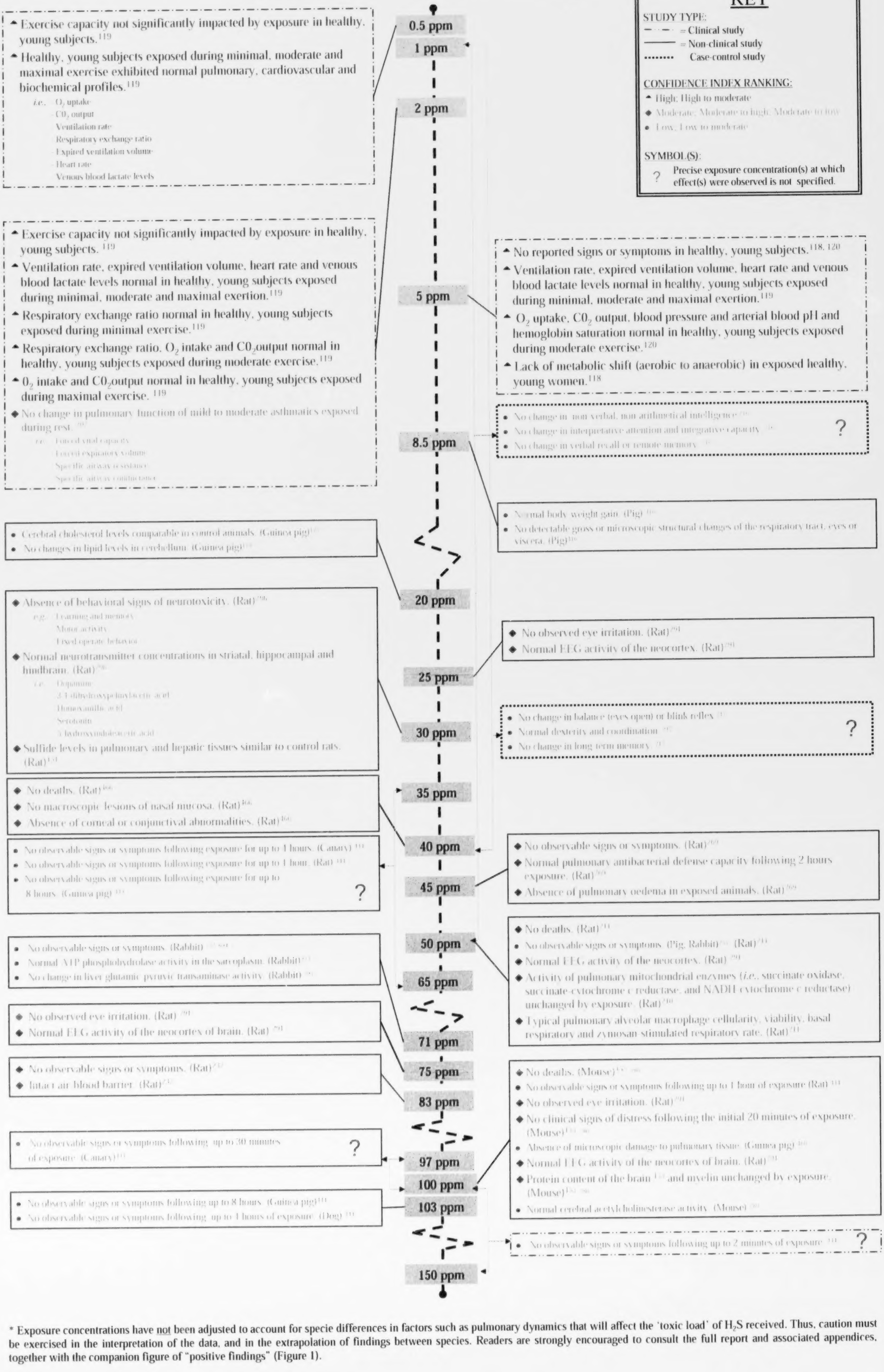
- ● Transient hyperactivity. (Mouse)
- ● Marked change in airway resistance and dynamic compliance consistent with bronchodilation. (Guinea pig)
- ● Marked dose related changes in hippocampal EEG activity. (Rat)
- ● Reduction in cerebral RNA synthesis. (Mouse)
- ● Increased activity of superoxide dismutase, 2', 3' cyclic nucleotide 3'phosphohydrolase in brain. (Mouse)
- ● Inhibition of brain cytochrome oxidase. (Mouse)
- ◆ Putative changes in brain neurotransmitter levels. (Rat)
  - *i.e.*, L-glutamate
- ● Transient alterations in protein metabolism in the brain. (Mouse)

- ● Death following more than 18 hours of exposure. (Rat)[111]
- ● Eye and nose irritation following more than 1 hour exposure. (Rat)[111] ?
- ● Forced breathing following more than 8 hours of exposure. (Rat)[111]

* Exposure concentrations have not been adjusted to account for specie differences in factors, such as pulmonary dynamics, that will affect the 'toxic load' of H_2S received. Thus, caution must be exercised in the interpretation of the data, and in the extrapolation of findings between species. Readers are strongly encouraged to consult the full report and associated appendices, together with the companion figure of "negative findings" (Figure 2).

Figure 2: Health effects associated with short-term low level exposure to H_2S.

Summary of "negative" findings.*



KEY

STUDY TYPE:
— · — · = Clinical study
——— = Non-clinical study
········ Case-control study

CONFIDENCE INDEX RANKING:
▲ High; High to moderate
◆ Moderate; Moderate to high; Moderate to low
• Low; Low to moderate

SYMBOL(S):
? Precise exposure concentration(s) at which effect(s) were observed is not specified.

- ▲ Exercise capacity not significantly impacted by exposure in healthy, young subjects.[119]
- ▲ Healthy, young subjects exposed during minimal, moderate and maximal exercise exhibited normal pulmonary, cardiovascular and biochemical profiles.[119]
  - *i.e.*, O_2 uptake
  - $C0_2$ output
  - Ventilation rate
  - Respiratory exchange ratio
  - Expired ventilation volume
  - Heart rate
  - Venous blood lactate levels

- ▲ Exercise capacity not significantly impacted by exposure in healthy, young subjects.[119]
- ▲ Ventilation rate, expired ventilation volume, heart rate and venous blood lactate levels normal in healthy, young subjects exposed during minimal, moderate and maximal exertion.[119]
- ▲ Respiratory exchange ratio normal in healthy, young subjects exposed during minimal exercise.[119]
- ▲ Respiratory exchange ratio, O_2 intake and $C0_2$output normal in healthy, young subjects exposed during moderate exercise.[119]
- ▲ 0_2 intake and $C0_2$output normal in healthy, young subjects exposed during maximal exercise.[119]
- ◆ No change in pulmonary function of mild to moderate asthmatics exposed during rest.[illegible]
  - *i.e.*, Forced vital capacity
  - Forced expiratory volume
  - Specific airway resistance
  - Specific airway conductance

- ▲ No reported signs or symptoms in healthy, young subjects.[118, 120]
- ▲ Ventilation rate, expired ventilation volume, heart rate and venous blood lactate levels normal in healthy, young subjects exposed during minimal, moderate and maximal exertion.[119]
- ▲ O_2 uptake, $C0_2$ output, blood pressure and arterial blood pH and hemoglobin saturation normal in healthy, young subjects exposed during moderate exercise.[120]
- ▲ Lack of metabolic shift (aerobic to anaerobic) in exposed healthy, young women.[118]

- • No change in non verbal, non arithmetical intelligence[illegible]
- • No change in interpretative attention and integrative capacity[illegible]
- • No change in verbal recall or remote memory[illegible]

?

- • Normal body weight gain. (Pig)[116]
- • No detectable gross or microscopic structural changes of the respiratory tract, eyes or viscera. (Pig)[116]

- • Cerebral cholesterol levels comparable to control animals. (Guinea pig)[illegible]
- • No changes in lipid levels in cerebellum. (Guinea pig)[illegible]

- ◆ Absence of behavioral signs of neurotoxicity. (Rat)[196]
  - *e.g.*, Learning and memory
  - Motor activity
  - Fixed operate behavior
- ◆ Normal neurotransmitter concentrations in striatal, hippocampal and hindbrain. (Rat)[196]
  - *i.e.*, Dopamine
  - 3,4-dihydroxyphenylacetic acid
  - Homovanillic acid
  - Serotonin
  - 5-hydroxyindoleacetic acid
- ◆ Sulfide levels in pulmonary and hepatic tissues similar to control rats. (Rat)[153]

- ◆ No observed eye irritation. (Rat)[291]
- ◆ Normal EEG activity of the neocortex. (Rat)[291]

- • No change in balance (eyes open) or blink reflex[illegible]
- • Normal dexterity and coordination[illegible]
- • No change in long term memory[illegible]

?

- ◆ No deaths. (Rat)[166]
- ◆ No macroscopic lesions of nasal mucosa. (Rat)[166]
- ◆ Absence of corneal or conjunctival abnormalities. (Rat)[166]

- • No observable signs or symptoms following exposure for up to 4 hours. (Canary)[111]
- • No observable signs or symptoms following exposure for up to 1 hour. (Rat)[111]
- • No observable signs or symptoms following exposure for up to 8 hours. (Guinea pig)[111]

?

- ◆ No observable signs or symptoms. (Rat)[269]
- ◆ Normal pulmonary antibacterial defense capacity following 2 hours exposure. (Rat)[269]
- ◆ Absence of pulmonary oedema in exposed animals. (Rat)[269]

- ◆ No deaths. (Rat)[211]
- • No observable signs or symptoms. (Pig, Rabbit)[illegible] (Rat)[211]
- ◆ Normal EEG activity of the neocortex. (Rat)[291]
- ◆ Activity of pulmonary mitochondrial enzymes (*i.e.*, succinate oxidase, succinate-cytochrome c reductase, and NADH cytochrome c reductase) unchanged by exposure. (Rat)[210]
- ◆ Typical pulmonary alveolar macrophage cellularity, viability, basal respiratory and zymosan-stimulated respiratory rate. (Rat)[211]

- • No observable signs or symptoms. (Rabbit)[illegible]
- • Normal ATP phosphohydrolase activity in the sarcoplasm. (Rabbit)[illegible]
- • No change in liver glutamic pyruvic transaminase activity. (Rabbit)[illegible]

- ◆ No observed eye irritation. (Rat)[291]
- ◆ Normal EEG activity of the neocortex of brain. (Rat)[291]

- ◆ No observable signs or symptoms. (Rat)[232]
- ◆ Intact air-blood barrier. (Rat)[232]

- • No observable signs or symptoms following up to 30 minutes of exposure. (Canary)[111]

?

- ◆ No deaths. (Mouse)[153, 196]
- • No observable signs or symptoms following up to 1 hour of exposure (Rat)[111]
- ◆ No observed eye irritation. (Rat)[291]
- ◆ No clinical signs of distress following the initial 20 minutes of exposure. (Mouse)[153, 196]
- • Absence of microscopic damage to pulmonary tissue. (Guinea pig)[illegible]
- ◆ Normal EEG activity of the neocortex of brain. (Rat)[291]
- ◆ Protein content of the brain[153] and myelin unchanged by exposure. (Mouse)[153, 196]
- • Normal cerebral acetylcholinesterase activity. (Mouse)[196]

- • No observable signs or symptoms following up to 8 hours. (Guinea pig)[111]
- • No observable signs or symptoms following up to 4 hours of exposure. (Dog)[111]

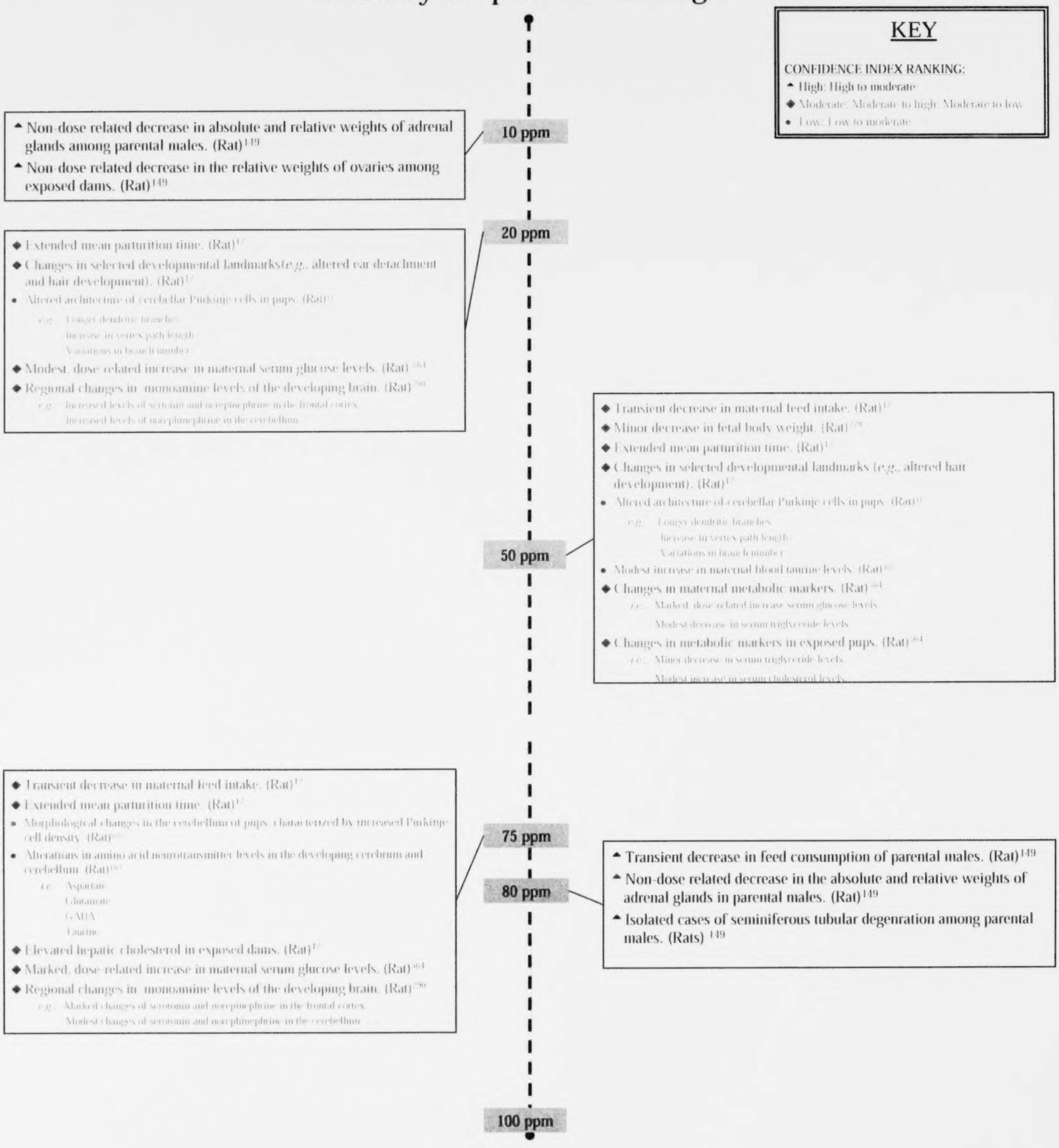
- • No observable signs or symptoms following up to 2 minutes of exposure[111]

?

* Exposure concentrations have not been adjusted to account for specie differences in factors such as pulmonary dynamics that will affect the 'toxic load' of H_2S received. Thus, caution must be exercised in the interpretation of the data, and in the extrapolation of findings between species. Readers are strongly encouraged to consult the full report and associated appendices, together with the companion figure of "positive findings" (Figure 1).

Figure 3: Health effects associated with low level exposure to H_2S during gestation.

Summary of "positive" findings.*



KEY

CONFIDENCE INDEX RANKING:

- ▲ High: High to moderate
- ◆ Moderate: Moderate to high; Moderate to low
- • Low: Low to moderate

10 ppm:

- ▲ Non-dose related decrease in absolute and relative weights of adrenal glands among parental males. (Rat)[149]
- ▲ Non-dose related decrease in the relative weights of ovaries among exposed dams. (Rat)[149]

20 ppm:

- ◆ Extended mean parturition time. (Rat)[17]
- ◆ Changes in selected developmental landmarks(*e.g.*, altered ear detachment and hair development). (Rat)[17]
- • Altered architecture of cerebellar Purkinje cells in pups. (Rat)[11]
  - *e.g.* Longer dendritic branches
  - Increase in vertex path length
  - Variations in branch number
- ◆ Modest, dose related increase in maternal serum glucose levels. (Rat)[364]
- ◆ Regional changes in monoamine levels of the developing brain. (Rat)[290]
  - *e.g.* Increased levels of serotonin and norepinephrine in the frontal cortex
  - Increased levels of norepinephrine in the cerebellum

50 ppm:

- ◆ Transient decrease in maternal feed intake. (Rat)[17]
- ◆ Minor decrease in fetal body weight. (Rat)[278]
- ◆ Extended mean parturition time. (Rat)[17]
- ◆ Changes in selected developmental landmarks (*e.g.*, altered hair development). (Rat)[17]
- • Altered architecture of cerebellar Purkinje cells in pups. (Rat)[11]
  - *e.g.* Longer dendritic branches
  - Increase in vertex path length
  - Variations in branch number
- • Modest increase in maternal blood taurine levels. (Rat)[160]
- ◆ Changes in maternal metabolic markers. (Rat)[364]
  - *i.e.* Marked, dose related increase serum glucose levels
  - Modest decrease in serum triglyceride levels
- ◆ Changes in metabolic markers in exposed pups. (Rat)[364]
  - *i.e.* Minor decrease in serum triglyceride levels
  - Modest increase in serum cholesterol levels

75 ppm:

- ◆ Transient decrease in maternal feed intake. (Rat)[17]
- ◆ Extended mean parturition time. (Rat)[17]
- • Morphological changes in the cerebellum of pups, characterized by increased Purkinje cell density. (Rat)[illegible]
- • Alterations in amino acid neurotransmitter levels in the developing cerebrum and cerebellum. (Rat)[180]
  - *i.e.* Aspartate
  - Glutamate
  - GABA
  - Taurine
- ◆ Elevated hepatic cholesterol in exposed dams. (Rat)[17]
- ◆ Marked, dose-related increase in maternal serum glucose levels. (Rat)[364]
- ◆ Regional changes in monoamine levels of the developing brain. (Rat)[290]
  - *e.g.* Marked changes of serotonin and norepinephrine in the frontal cortex
  - Modest changes of serotonin and norepinephrine in the cerebellum

80 ppm:

- ▲ Transient decrease in feed consumption of parental males. (Rat)[149]
- ▲ Non-dose related decrease in the absolute and relative weights of adrenal glands in parental males. (Rat)[149]
- ▲ Isolated cases of seminiferous tubular degenration among parental males. (Rats) [149]

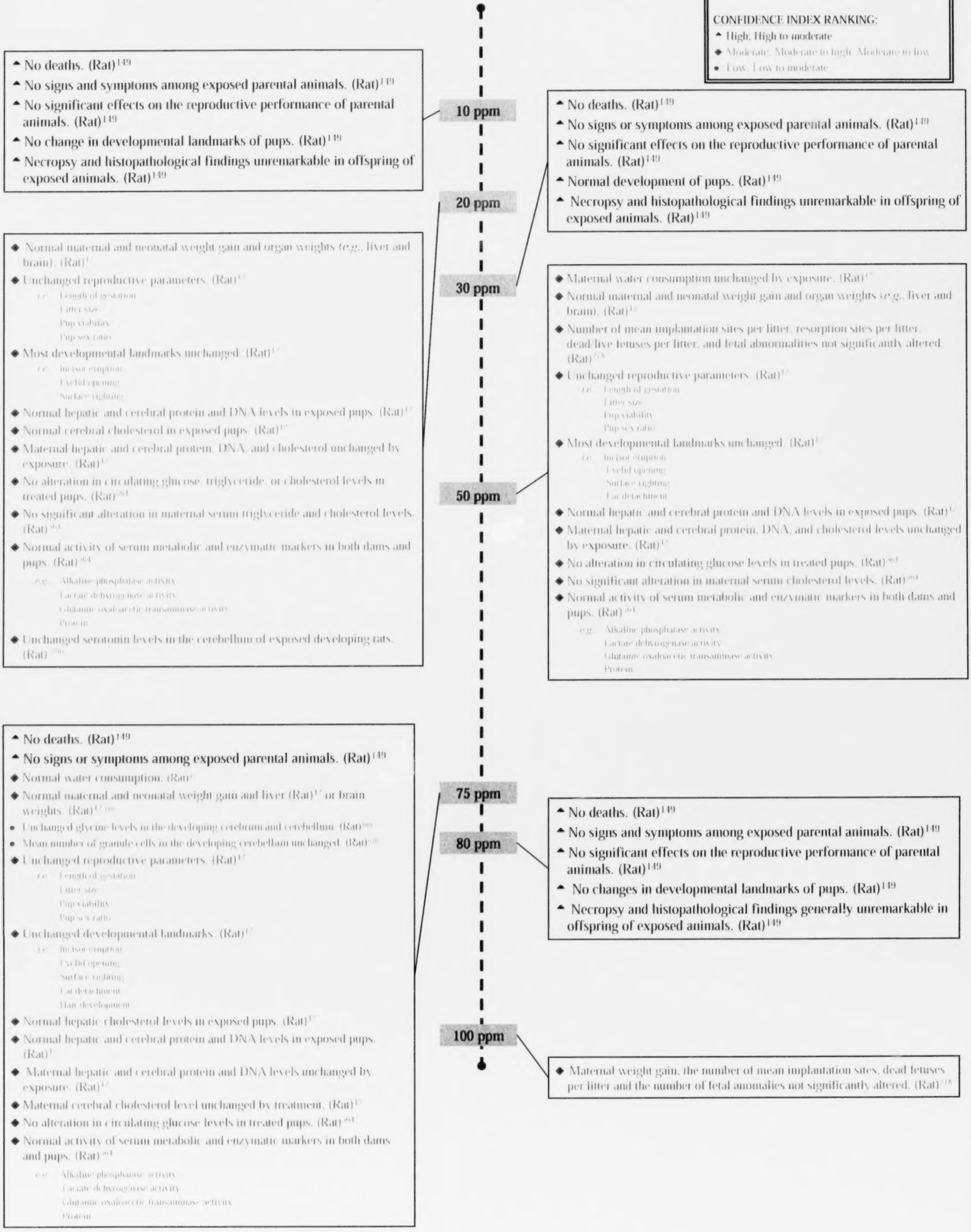
*** Exposure concentrations have not been adjusted to account for specie differences in factors, such as pulmonary dynamics, that will affect the 'toxic load' of H_2S received. Thus, caution must be exercised in the interpretation of the data, and in the extrapolation of findings between species. Readers are strongly encouraged to consult the full report and associated appendices, together with the companion figure of "negative findings" (Figure 4).**

Figure 4: Health effects associated with low level exposure to H_2S during gestation.

Summary of "negative" findings.*

KEY

CONFIDENCE INDEX RANKING:
- ▲ High; High to moderate
- ◆ Moderate; Moderate to high; Moderate to low
- • Low; Low to moderate



- ▲ No deaths. (Rat)[149]
- ▲ No signs and symptoms among exposed parental animals. (Rat)[149]
- ▲ No significant effects on the reproductive performance of parental animals. (Rat)[149]
- ▲ No change in developmental landmarks of pups. (Rat)[149]
- ▲ Necropsy and histopathological findings unremarkable in offspring of exposed animals. (Rat)[149]

- ◆ Normal maternal and neonatal weight gain and organ weights (*e.g.*, liver and brain). (Rat)[17]
- ◆ Unchanged reproductive parameters. (Rat)[17]
  - *i.e.* Length of gestation
  - Litter size
  - Pup viability
  - Pup sex ratio
- ◆ Most developmental landmarks unchanged. (Rat)[17]
  - *i.e.* Incisor eruption
  - Eyelid opening
  - Surface righting
- ◆ Normal hepatic and cerebral protein and DNA levels in exposed pups. (Rat)[17]
- ◆ Normal cerebral cholesterol in exposed pups. (Rat)[17]
- ◆ Maternal hepatic and cerebral protein, DNA, and cholesterol unchanged by exposure. (Rat)[17]
- ◆ No alteration in circulating glucose, triglyceride, or cholesterol levels in treated pups. (Rat)[164]
- ◆ No significant alteration in maternal serum triglyceride and cholesterol levels. (Rat)[164]
- ◆ Normal activity of serum metabolic and enzymatic markers in both dams and pups. (Rat)[164]
  - *e.g.* Alkaline phosphatase activity
  - Lactate dehyrogenase activity
  - Glutamic oxaloacetic transaminase activity
  - Protein
- ◆ Unchanged serotonin levels in the cerebellum of exposed developing rats. (Rat)[230]

- ▲ No deaths. (Rat)[149]
- ▲ No signs or symptoms among exposed parental animals. (Rat)[149]
- ▲ No significant effects on the reproductive performance of parental animals. (Rat)[149]
- ▲ Normal development of pups. (Rat)[149]
- ▲ Necropsy and histopathological findings unremarkable in offspring of exposed animals. (Rat)[149]

- ◆ Maternal water consumption unchanged by exposure. (Rat)[17]
- ◆ Normal maternal and neonatal weight gain and organ weights (*e.g.*, liver and brain). (Rat)[17]
- ◆ Number of mean implantation sites per litter, resorption sites per litter, dead/live fetuses per litter, and fetal abnormalities not significantly altered. (Rat)[178]
- ◆ Unchanged reproductive parameters. (Rat)[17]
  - *i.e.* Length of gestation
  - Litter size
  - Pup viability
  - Pup sex ratio
- ◆ Most developmental landmarks unchanged. (Rat)[17]
  - *i.e.* Incisor eruption
  - Eyelid opening
  - Surface righting
  - Ear detachment
- ◆ Normal hepatic and cerebral protein and DNA levels in exposed pups. (Rat)[17]
- ◆ Maternal hepatic and cerebral protein, DNA, and cholesterol levels unchanged by exposure. (Rat)[17]
- ◆ No alteration in circulating glucose levels in treated pups. (Rat)[164]
- ◆ No significant alteration in maternal serum cholesterol levels. (Rat)[164]
- ◆ Normal activity of serum metabolic and enzymatic markers in both dams and pups. (Rat)[164]
  - *e.g.* Alkaline phosphatase activity
  - Lactate dehyrogenase activity
  - Glutamic oxaloacetic transaminase activity
  - Protein

- ▲ No deaths. (Rat)[149]
- ▲ No signs or symptoms among exposed parental animals. (Rat)[149]
- ◆ Normal water consumption. (Rat)[17]
- ◆ Normal maternal and neonatal weight gain and liver (Rat)[17] or brain weights. (Rat)[17,180]
- • Unchanged glycine levels in the developing cerebrum and cerebellum. (Rat)[180]
- • Mean number of granule cells in the developing cerebellum unchanged. (Rat)[230]
- ◆ Unchanged reproductive parameters. (Rat)[17]
  - *i.e.* Length of gestation
  - Litter size
  - Pup viability
  - Pup sex ratio
- ◆ Unchanged developmental landmarks. (Rat)[17]
  - *i.e.* Incisor eruption
  - Eyelid opening
  - Surface righting
  - Ear detachment
  - Hair development
- ◆ Normal hepatic cholesterol levels in exposed pups. (Rat)[17]
- ◆ Normal hepatic and cerebral protein and DNA levels in exposed pups. (Rat)[17]
- ◆ Maternal hepatic and cerebral protein and DNA levels unchanged by exposure. (Rat)[17]
- ◆ Maternal cerebral cholesterol level unchanged by treatment. (Rat)[17]
- ◆ No alteration in circulating glucose levels in treated pups. (Rat)[164]
- ◆ Normal activity of serum metabolic and enzymatic markers in both dams and pups. (Rat)[164]
  - *e.g.* Alkaline phosphatase activity
  - Lactate dehyrogenase activity
  - Glutamic oxaloacetic transaminase activity
  - Protein

- ▲ No deaths. (Rat)[149]
- ▲ No signs and symptoms among exposed parental animals. (Rat)[149]
- ▲ No significant effects on the reproductive performance of parental animals. (Rat)[149]
- ▲ No changes in developmental landmarks of pups. (Rat)[149]
- ▲ Necropsy and histopathological findings generally unremarkable in offspring of exposed animals. (Rat)[149]

- ◆ Maternal weight gain, the number of mean implantation sites, dead fetuses per litter and the number of fetal anomalies not significantly altered. (Rat)[178]

* **Exposure concentrations have not been adjusted to account for specie differences in factors, such as pulmonary dynamics, that will affect the 'toxic load' of H_2S received. Thus, caution must be exercised in the interpretation of the data, and in the extrapolation of findings between species. Readers are strongly encouraged to consult the full report and associated appendices, together with the companion figure of "positive findings" (Figure 3).**

APPENDIX 1

TITLES AND AFFILIATIONS OF THE EXPERT PANEL

APPENDIX 1: TITLES AND AFFILIATIONS OF THE EXPERT PANEL

Randy P. Angle, PhD
Assistant Director, Air & Water Section
Science & Standards Branch
Environmental Assurance Division, Alberta Environment

Nicholas J. Bayliss, MB, BSc, MPH
Associate Provincial Health Officer
Alberta Health and Wellness

Donald B. Davies, PhD, DABT
Vice-President, Scientific Programs
Cantox Environmental Inc.

Brent Friesen, MD, FRCPC
Medical Officer of Health
Calgary Health Region
Council of Medical Officers of Health

Stephan Gabos, MD
Director
Health Surveillance
Health Strategies Division
Alberta Health and Wellness

Geoffrey C. Granville
Manager, Toxicology & Product Stewardship
Shell Canada Limited

Paul Hasselback MD, MSc, FRCPC
Vice-President/Medical Officer of Health
Chinook Health Region

Alex MacKenzie
Health Assessment Coordinator
Health Surveillance
Health Strategies Division
Alberta Health and Wellness

Ingrid Vicas, MSc, MDCM, FRCPC, FACMT
Director/ Medical Director Poison & Drug Information Service (Alberta)

Medical Toxicologist, Alberta Poison Centre
Calgary Health Region

APPENDIX 2

SEARCH STRATEGY

APPENDIX 2: SEARCH STRATEGY

	Inclusion/Exclusion terms:	Number of documents identified:
1. Title or keywords must INCLUDE at least one of the following terms:	H_2S; Hydrogen sulfide; Hydrogen sulphide; Sour gas	9715
2. From the list generated by (1), INCLUDE documents with any of the following terms in the title or keywords:	Acute; Subacute; Short term; Accident; Poison; Case stud-; Toxic; Adverse; Neurolog; CNS; Central nervous system; nervous; brain; behavior; Clinical; Clinical trial; Occup-; Teratol; Teratogen; Embryotox; Reproduct-; Development; Pregnan-; Fetus; Fetal; Birth Defect	3538
3. From the list generated by (2), EXCLUDE documents with any of the following terms in the title or keywords:	Cancer; Carcinogen; Tumour; Tumor; Neoplas-; Oncogen; Adenoma; Malignan-; Genotox; Mutat-; Mutagen; Cytogen; Cytotox; Clastogen; DNA repair; Aquatic; Marine; Benthic; Fish; Invertebrate; Periodent; Peridont; Endogenous; Remov-; Corrosion; Intensificat-; Cataly-; Scaveng-; Rice; Computer; Iron; Steel; Nickel; Alloy; Spinach	1461
4. From the list generated by (3), ISOLATE by publication language:	English only	1169

APPENDIX 3

SOURCE CITATIONS

APPENDIX 3: SOURCE CITATIONS

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(STUDY ID-328)

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Argo, J. 1998. Retrospective exposure assessment with emission inventories: A new approach to an old problem. Environmetrics 9: 505-518.
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(STUDY ID-523)

Lee, D.W., Prior, M.G., and Toma, D.S. 1986. Toxicology of Sulphur Gases: A Bibliography. Supplement 1. Alberta Environmental Centre. Vegreville, Alberta. 128 p. ISSN 082803052. AECV85-L1.
(STUDY ID-764)

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(STUDY ID-763)

Lopez, A., Prior, M., Leblanc, D., Young, S., Al-Bassam, M., and Lillie, L.E. 1986. Morphological observations in rats exposed for six hours to an atmosphere of 0, 56, or 420 mg/m^3 hydrogen sulfide. Alberta Environmental Centre. Vegreville, AB. AECV86-A1: p 1-28.
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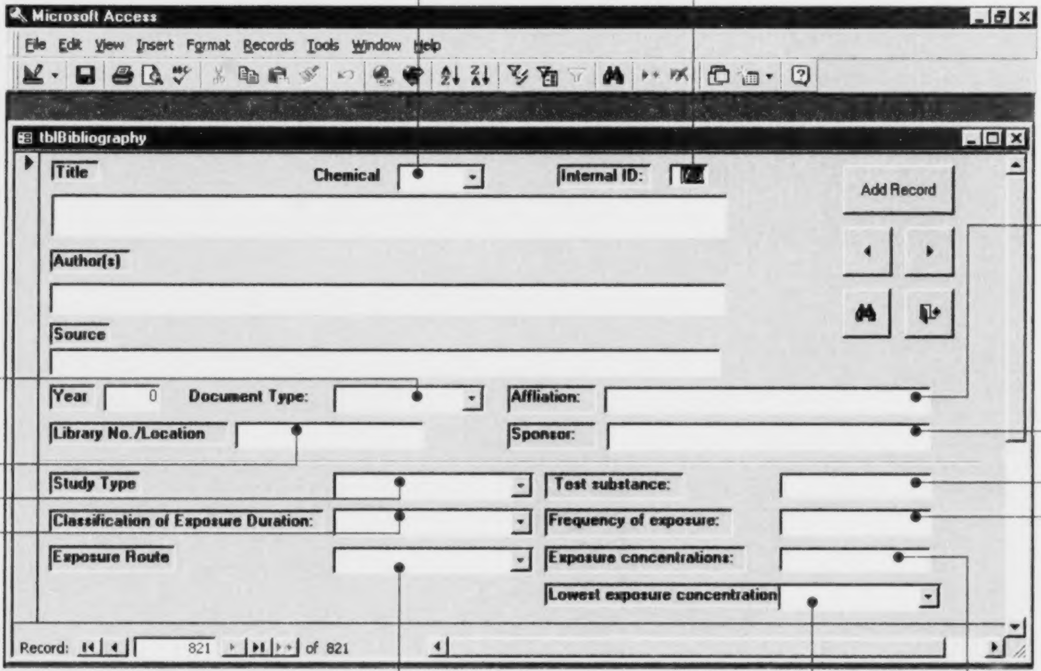
(STUDY ID-254)

USEPA. 1993. Health assessment document for hydrogen sulfide. http://www.epa.gov/cpi-bin/claritgw
(STUDY ID-332)

APPENDIX 4

DATABASE BIBLIOGRAPHICAL FORM

APPENDIX 4: DATABASE BIBLIOGRAPHICAL FORM


Microsoft Access
File Edit View Insert Format Records Tools Window Help
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Title
Chemical
Internal ID:
Add Record
Author(s)
Source
Year
0
Document Type:
Affliation:
Library No./Location
Sponsor:
Study Type
Test substance:
Classification of Exposure Duration:
Frequency of exposure:
Exposure Route
Exposure concentrations:
Lowest exposure concentration
Record:
821
of 821

Chemical: *e.g.*, H_2S, $S0_2$ or General.

Document type: *e.g.*, Report, periodical, abstract, brochure, etc.

Library No./Location: Cantox Environmental Inc. internal study ID.

Study type: *e.g.*, Clinical, non-clinical, epidemiology, case-study, review, etc.

Classification of exposure duration: *e.g.*, Acute, subacute, subchronic, chronic, etc.

Internal ID: Document identification number within database.

Affiliation: The affiliation (*e.g.*, organization, company) of the author(s).

Sponsor: The source of funding.

Test substance: The exact chemical formulation to which receptor was exposed.

Frequency of exposure: *e.g.*, single or repeated exposure.

Exposure route: The route of exposure (*e.g.*, inhalation, ingestion, dermal, etc.)

Lowest exposure concentration: The concentration range containing the lowest exposure concentration to which test receptors were exposed.
[NB: 0-1ppm = < 1ppm]

Exposure concentration(s): The exposure concentration as noted in the text.

APPENDIX 5

A: CASE-CONTROL REVIEW FORM

B: CLINICAL REVIEW FORM

C: NON-CLINICAL REVIEW FORM

APPENDIX 5A: CASE-CONTROL REVIEW FORM

Author:		**Internal ID:**	ID of study within database
Title:			
Year:			
Abstract:			
Objective:	The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
						No observed adverse effect level	Lowest observed adverse effect level

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions.

Review – Assessment: **[NB: In actual reviews ☐ is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]**

A. Subjects:	☐ Is the number of subjects sufficient to produce statistically significant results? ☐ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ☐ Are the subjects a representative sample of the population to which extrapolations will be made? ☐ Were identical 'criteria for exclusion' applied to both cases and controls? ☐ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	☐ Was the exposure concentration measured? ☐ Is the exposure duration known?
C. Equipment:	☐ Was a full description of the monitoring devices provided? ☐ What was the sensitivity of the monitoring devices employed?

D. Procedural:	☐ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? ☐ Were appropriate controls recruited? ☐ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ☐ Was the timing and length of follow-up appropriate? ☐ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	☐ Was the assessment identical for cases and controls? ☐ Were appropriate endpoints examined? ☐ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ☐ Were accepted assessment methods employed? ☐ Were assessment measures sufficiently sensitive? ☐ Was the nature and severity of symptoms graded against an explicitly defined scale? ☐ Did qualified personnel conduct the exams? ☐ Was the timing and frequency of assessment logical? ☐ Was raw data provided to permit the reader to arrive at his/her own conclusions? ☐ Was individual data reported for each subject? ☐ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	☐ Are the statistical methods used appropriate? ☐ Are confidence intervals reported? ☐ Did the investigator appropriately interpret the statistics? ☐ Are the observed trends statistically significant?
G. Interpretations:	☐ Was the original objective addressed? ☐ Are the results novel? ☐ Was the design of the study appropriate to investigate the primary objective? ☐ Were assumptions reasonable? ☐ Are the methods reproducible? ☐ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? ☐ Do the conclusions make biological sense? ☐ Are the extrapolations reasonable? ☐ Is the paper in a peer-reviewed journal? ☐ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.

Confidence index:

Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

High
High to moderate
Moderate to high
Moderate
Moderate to low
Low to moderate
Low

APPENDIX 5B: CLINICAL REVIEW FORM

Author:		**Internal ID:**	ID of study within database
Title:			
Year:			
Abstract:			
Objective:	The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
						No observed adverse effect level	Lowest observed adverse effect level

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions.

Review – Assessment: **[NB: In actual reviews ☐ is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]**

A. Subjects:	☐ Is the number of subjects sufficient to produce statistically significant results? ☐ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ☐ Are the subjects a representative sample of the population to which extrapolations will be made? ☐ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ☐ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	☐ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? ☐ Was there a gradient of exposure levels? ☐ Were the chosen exposure levels appropriate to investigate the primary objective? ☐ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ☐ Were both nominal and actual exposure concentrations recorded? ☐ Was the duration of exposure appropriate to investigate the primary objective? ☐ Was the duration of exposure precisely defined? ☐ Was the mode of administration appropriate?

	☐ Was the frequency of administration appropriate to investigate the primary objective? ☐ Was evidence provided to justify the dosing regime? ☐ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	☐ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined ☐ Was a full description of the monitoring devices provided? ☐ What was the sensitivity of the monitoring devices employed?
D. Procedural:	☐ Were the subjects randomly assigned to exposure groups? ☐ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) ☐ Was a control group assayed? ☐ Is the control regime appropriate? ☐ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ☐ Was the length of follow-up appropriate? ☐ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	☐ Were appropriate endpoints examined? ☐ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ☐ Were accepted assessment methods employed? ☐ Were assessment measures sufficiently sensitive? ☐ Was the nature and severity of symptoms graded against an explicitly defined scale? ☐ Did qualified personnel conduct the exams? ☐ Was the timing and frequency of assessment logical? ☐ Was raw data provided to permit the reader to arrive at his/her own conclusions? ☐ Was individual data reported for each subject? ☐ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	☐ Are the statistical methods used appropriate? ☐ Are confidence intervals reported? ☐ Did the investigator appropriately interpret the statistics? ☐ Are the observed trends statistically significant?
G. Interpretations:	☐ Was the original objective addressed? ☐ Are the results novel? ☐ Was the design of the study appropriate to investigate the primary objective? ☐ Were assumptions reasonable? ☐ Are the methods reproducible? ☐ Did either the study design or the statistical analysis limit confounding variables? ☐ Do the conclusions make biological sense? ☐ Are the extrapolations reasonable? ☐ Is the paper in a peer-reviewed journal? ☐ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.

Confidence index:

Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

High
High to moderate
Moderate to high
Moderate
Moderate to low
Low to moderate
Low

APPENDIX 5C: NON-CLINICAL REVIEW FORM

Author:		**Internal ID:**	ID of study within database
Title:			
Year:			
Abstract:			
Objective:	The authors stated objective and identification of relevant experiments in documents reporting findings from multiple studies.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
								No observed adverse effect level	Lowest observed adverse effect level

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions.

Review – Assessment: **[NB: In actual reviews □ is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]**

A. Test animals:	☐ Is the number of animals sufficient to produce statistically significant results? • *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ☐ Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie • *Guideline: Rat, or justification for use of another mammalian specie.* b) Age • *Guideline: Adult, i.e.,8-12 weeks.* c) Sex d) Pre-test health • *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation • *Guideline: Below 20% variation* f) Source

	• *Guideline: An established breeding facility or educational institution.*
B. Exposure conditions:	☐ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? ☐ Was there a gradient of exposure levels? • *Guideline: Three exposure levels in addition to control.* ☐ Were the chosen exposure levels appropriate to investigate the primary objective? ☐ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ☐ Were both nominal and actual exposure concentrations recorded? ☐ Was the duration of exposure appropriate to investigate the primary objective? ☐ Was the duration of exposure precisely defined? ☐ If exposure chambers were employed, were animals exposed during the equilibration period? • *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ☐ Was the mode of administration appropriate? ☐ Was the frequency of administration appropriate to investigate the primary objective? ☐ Was evidence provided to justify the dosing regime? ☐ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	☐ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? • *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ☐ Was the number of animals grouped in a single chamber stated? • *Guideline: Groupings should permit clear observation of each animal.* ☐ Was the manner by which animals were grouped appropriate? • *Guideline: Animals may be grouped by sex, or individually.* ☐ Was the type and source of feed and water stated? Was it appropriate? • *Guideline: Conventional lab diet* ☐ Was the amount and feeding schedule specified? • *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	☐ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. • *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ☐ Was a full description of the monitoring devices provided? ☐ What was the sensitivity of the monitoring devices employed?
E. Procedural:	☐ Was the acclimation period specified? Was it of acceptable duration? • *Guideline: 5 days* ☐ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ☐ Were test animals randomly assigned to exposure groups? ☐ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) ☐ Was a control group assayed? ☐ Is the control regime appropriate? ☐ Was the period of observation following exposure appropriate? • *Guideline: Longer than 14 days.*

F. Data collection:	☐ Were appropriate endpoints examined? ☐ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? ☐ Were accepted assessment methods employed? ☐ Were assessment measures sufficiently sensitive? ☐ Was the nature and severity of symptoms graded against an explicitly defined scale? ☐ Did qualified personnel conduct the exams? ☐ Was the timing and frequency of assessment logical? ☐ Was raw data provided to permit the reader to arrive at his/her own conclusions? ☐ Was individual data reported for each test animal?
G. Data analysis:	☐ Are the statistical methods used appropriate? ☐ Are confidence intervals reported? ☐ Did the investigator appropriately interpret the statistics? ☐ Are the observed trends statistically significant?
H. Interpretations:	☐ Was the original objective addressed? ☐ Are the results novel? ☐ Was the design of the study appropriate to investigate the primary objective? ☐ Were assumptions reasonable? ☐ Are the methods reproducible? ☐ Did either the study design or the statistical analysis limit confounding variables? ☐ Do the conclusions make biological sense? ☐ Are the extrapolations reasonable? ☐ Is the paper in a peer-reviewed journal? ☐ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.

Confidence index:

Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

High
High to moderate
Moderate to high
Moderate
Moderate to low
Low to moderate
Low

APPENDIX 6

DETAILED DOCUMENT REVIEWS

Document Review

Author:	Hannah, RS; Roth, SH	**Internal ID:**	11
Title:	Chronic exposure to low concentrations of hydrogen sulfide produces abnormal growth in developing cerebellar Purkinje cells.		
Year:	1991		
Abstract:	*"Hydrogen sulfide (H_2S) may produce deleterious effects on the developing central nervous system. The dendritic fields of developing cerebellar Purkinje cells were analyzed to determine the effects of chronic exposure to low concentrations of H_2S during perinatal development. Treatment with two concentrations (20 and 50ppm) of H_2S produced severe alterations in the architecture and growth characteristics of the Purkinje cell dendritic fields. The architectural modifications included longer branches, an increase in the vertex path length and variations in the number of branches in particular areas of the dendritic field. The treated cells also exhibited a nonsymmetrical growth pattern at a time when random terminal branching is normally occurring. These findings suggest that developing neurons exposed to low concentrations of H_2S are at risk of severe deficits."*		
Objective:	To examine the morphological effects of exposure to low concentrations of H_2S on the developing nervous system of the rat. (The "dendritic tree of cerebellar Purkinje cells" was used as a representative model to study the morphological effects).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 20ppm; 50ppm	Repeated/ 7 hours per day from day 5 postcoitus until day 21 postnatal.	Rat	Sprague-Dawley	Not specified	Female. Sex of pups was not specified.	40 litters. (10 exposed to 20ppm; 10 exposed to 50ppm; 20 control)	Timed-pregnant. Pup health was not evaluated.	NA	20ppm (?)

Observations:

- Mean dendritic segment length:
    - 20ppm: Inconsistent results.
    - 50ppm: Longer over the low and middle "orders" (*i.e.*, orders 1 to 18).*
    - Visual examination of the findings (*i.e.*, Figure 2 of report) did not reveal a clear dose-related response.

- Mean terminal path length (mean length from tips of all terminal dendrites back to the axon hillock):
    - 20ppm: 7% increase*
    - 50ppm: 8% increase*

- Mean vertex path length (mean distance from any one equivalent order back to the axon hillock):
    - Distance between successive orders: 12% higher than controls.*
    - No indication of dose-responsiveness based on visual examination of findings (*i.e.*, Figure 3 of report).

- Segment frequency (mean number of branches at each equivalent order):
    - Increase in branch number over the middle orders (*i.e.*, 8-14) and a decrease in numbers over the higher orders (*i.e.*, 15-25).
    - Total segment frequency unchanged (*i.e.*, across all orders).
    - No indication of dose-responsiveness.

- Ratio of the types of branch points (Va/Vb):
  - 20ppm: 56% increase.
  - 50ppm: 66% increase.

*Statistically significant

Author's Conclusions:

- *"Treatment with two concentrations (20 and 50ppm) H_2S produced severe alterations in the architecture and growth characteristics of the Purkinje cell dendritic fields. The architectural modifications included longer branches, an increase in the vertex path length and variations in the number of branches in particular areas of the dendritic field."*
- *"Treated cells also exhibited a nonsymmetrical growth pattern at a time when random terminal branching is normally occurring."*
- *"...findings suggest that developing neurons exposed to low concentrations of H_2S are at risk of severe deficits."*

Review – Assessment:

A. Test animals:	- The number of dams on test was not reported. It was only indicated that 10 litters were selected from each of the various control and treated groups. - The use of 10 litters as the unit of comparison does not meet guideline recommendations for the general study of developmental toxicity. Guidelines typically recommend use of at least 20 litters per dose group. + The strain and specie of test animals was reported. - The pre-test health, age and source of the dams were not specified. - The sex of pups selected for examination was not noted.
B. Exposure conditions:	- The source of the H_2S gas was not reported. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether the exposure concentration was allowed to equilibrate before the animals were placed into the chamber. +/- No description of the exposure chamber or gas delivery system was supplied. Details were provided in a companion paper (Hannah *et al.*, 1989 – Study ID 180). - Chamber conditions could not be verified.
C. Housing/Feeding:	- Details concerning animal housing were lacking. - It is unclear as to whether the dams and litters were housed in the exposure chamber during the entire treatment period, or whether the animals were moved in and out of the chamber from conventional housing for the 7-hour exposure period each day. - Details concerning environmental conditions within the animal room (*i.e.*, temperature, humidity, photoperiod) were not supplied. - Bedding and nesting materials were not specified. + Dams were allowed to eat and drink *ad libitum* except during the exposure period. This feeding schedule complies with guideline recommendations. - The source and type of feed was not given. - Details concerning the water supply were not provided.
D. Equipment:	+/- Details concerning exposure equipment were lacking. Specifics concerning equipment were available in a companion paper, but the details could not be verified.
E. Procedural:	- No acclimatization period was reported.

	- No description of pre-test conditions was provided. +/- Pups were randomly chosen for examination, but whether dams were randomly assigned to exposure groups was not noted. Details concerning the method of randomization (*e.g.* table of random numbers, computer generated, etc.) were not supplied. + Control group of animals (0 ppm) was included and control animals were maintained in the same conditions as the treated group, but without H_2S exposure. +/- It would appear that the study consisted of 2 separate exposure trials (*i.e.*, 20 and 50 ppm), each with a separate control group. (This confounds interpretation of the study findings, which were combined for reporting purposes). +/- Unclear whether technicians and handlers were blinded to exposure conditions. - Analysis was conducted on only on one cell sample from one pup per litter. - Conventional measures of toxicity (*i.e.*, clinical observations, body weights, pregnancy outcome, litter parameters, gross pathology) were not performed. - No indication that study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- *"Fully impregnated Purkinje cells were selected…and drawn using a camera lucida at a magnification of x1400…The drawings were placed on a digitizer and vertex analysis performed according to the method of Berry and Finn."*. The validity of this method of analysis is unknown. - Individual data were not provided for each test animal, thereby, limiting the independent assessment of the findings. - No raw data was reported. Results were only displayed graphically, and resolution was generally lacking. - The reversibility of the observed changes was not investigated.
G. Data analysis:	+ Statistical evaluation of all data was carried out with the Student's *t* test. - The statistical significance of some parameters was not stated (*i.e.*, segment frequency, ratio of types of branch points). - Confidence intervals were not provided.
H. Interpretation:	- Conclusions were based largely on hypotheses, without support of empirical data. - Dose-response relationships were not obvious for most parameters measured. Despite a 2.5-fold increase in exposure (*i.e.*, 20 ppm to 50 ppm), differences in responses between treatment groups was marginal. - Interpretation of findings is confounded by experimental design, which evidently involved 2 separate exposure trials.

Review - Summary:

Discussion of findings:

Changes in the morphological architecture of cerebellar Purkinje cells were examined in the offspring of pregnant female rats following exposure to 0, 20, or 50 ppm H_2S from Day 5 post-coitus until Day 21 post-natal. Changes in dendritic segment length, terminal path length, vertex path length, segment frequency, and ratio of types of branch points were described in Purkinje cells isolated from the treated pups relative to controls. The changes suggested abnormal development at the *"growth front"* of the cells, characterized by non-symmetrical growth.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- Although changes in the morphological architecture of the Purkinje cells were demonstrated at exposure concentrations of H_2S as low as 20 ppm,, the toxicological significance of the findings is questionable. A lack of dose-responsiveness for the majority of changes observed renders interpretation of the significance of the findings difficult. Specifically, a clear indication of dose-responsiveness was not evident for the changes in branch length, terminal path length, vertex path length, and segment frequency across most orders. Despite a 2.5-fold increase in exposure concentration, the differences in these responses were only marginal among the pups exposed to 20 ppm H_2S and those exposed to 50 ppm. For some responses, the differences were practically non-discernable owing, in part, to the lack of resolution afforded by the graphical presentation of the data. Interpretation might have been improved through tabulation of the findings.
- The significance of the findings might have been strengthened by the use of a greater number of test animals and examination of more litters. The use

of only 10 litters per exposure concentration, and the examination of only a single Purkinje cell from a single pup from each litter were judged to limit the usefulness of the study. Most testing guidelines aimed at the study of developmental effects recommend the use of 20 dams per treatment group and the examination of *all* litters and *all* pups, with provision for the culling of litters to 8 pups on Day 4 post-partum to ease stress on the mother and to reduce inter-litter variability.

- Regrettably, no information concerning the clinical state of the treated dams or pups during the exposures was available. Such information for the pups could have improved the usefulness and relevance of the study since the changes in dendritic architecture might have been correlated with abnormal clinical signs. In addition, the lack of such information for the dams precludes any consideration as to whether the reported changes in dendritic architecture represent direct toxicity of H_2S on the developing nervous system or secondary responses to maternal stress. Typically, responses that can be traced to maternal stress are assigned less significance than changes that represent direct toxicity.
- In assessing the relevance of the observed changes, some consideration should be given to the nature of the exposure regimen. The dams and/or pups were exposed to 20 or 50 ppm of H_2S for 7 hours per day for up to 38 days. Changes were not assessed until Day 21 post-partum. The equivalent exposure time for humans would be significantly greater, extending over several months. The likelihood of occurrence of human exposures matching those examined in the study should be considered when judging the relevance of the reported changes in dendritic architecture.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to, undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of too few animals.
- Reliance on examination of a single Purkinje cell from a single pup from each litter selected for study.
- Lack of information respecting disposition of dams and selection of litters for study.
- Failure to perform treatment exposures concurrently (*i.e.*, it would appear that two separate exposure trials were performed).
- General lack of information concerning test animals, housing conditions, and the exposure system.
- Presentation of results exclusively in the form of graphs and figures of limited resolution, with no numerical findings provided.
- Failure to include any conventional measures of toxicity as part of the study.

Review – Confidence index ranking:

Low to moderate

Document Review

Author:	Hayden, LJ; Goeden, H; Roth, SH	**Internal ID:**	12
Title:	Growth and development in the rat during sub-chronic exposure to low levels of hydrogen sulfide.		
Year:	1990		
Abstract:	*"The effects of low levels of hydrogen sulfide (H_2S) on mammalian growth and development are unknown although it has long been postulated that H_2S can inhibit critical developmental functions through the cleavage of disulfide bonds and chelation of essential metal ions. Gravid rat dams exposed to H_2S ($\leq$75 PPM) from day 6 of gestation until day 21 postpartum (PP) demonstrated normal reproductive parameters until parturition. At parturition, however, delivery time was extended in a dose dependent manner with a maximum increase of 42% at 75 PPM. Maternal liver cholesterol content was elevated significantly on day 21 postpartum following exposure to 75 PPM H_2S each day for 6 weeks. Pups which were exposed in utero and neonatally to day 21 postpartum developed with a subtle decrease in time of ear detachment and hair development and with no other observed change in growth and development through day 21 postpartum."*		
Objective:	To investigate the impact of exposure to $\leq$75ppm H_2S, from day 6 of gestation through to day 21 postpartum, on maternal health and on the growth and development of the conceptus and neonate.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 20ppm; 50ppm; 75ppm[1]	Repeated/ 7 hours per day from Day 6 of gestation until Day 21 postpartum.	Rat	Sprague-Dawley	Parental males and females were 80-90 days of age when mated.	Both	10 gravid females per exposure concentration. Number of pups examined varied.	Not specified.	NA	20ppm

1. From the description of methods contained in the report, it would appear that a series of experiments were performed, each with a single exposure concentration and separate control group. According to the report, two separate experiments involving exposures to 20 ppm were conducted.

Observations:

- Maternal weight gain: Similar for both control and treated dams.

- Feed consumption:
  - 50ppm:
    - Day 5-9: 11% decrease relative to controls*
    - Day 9-13: 5% increase
    - Day 13-17: 7% increase
    - Day 17-21: 4% increase
  - 75ppm:
    - Day 5-9: 14% decrease*
    - Day 9-13: 7% decrease*
    - Day 13-17: 3% decrease
    - Day 17-21: 1% decrease

- Water consumption:
    - 50ppm:
        - Day 5-9: 8% decrease relative to controls
        - Day 9-13: 2% decrease
        - Day 13-17: 3% increase
        - Day 17-21: 2% decrease
    - 75ppm:
        - Day 5-9: 3% decrease
        - Day 9-13: 6% increase
        - Day 13-17: 3% decrease
        - Day 17-21: 3% decrease

- Maternal organ weights:
    - Liver weight: No significant change relative to controls
    - Brain weight: No significant change

- Developmental and reproductive data:
    - Gestation length: No significant change
    - Litter size: No significant change
    - Viability: No significance change
    - Male/Female ratio: No significant change
    - Pinna detachment (number of days):
        - 20ppm: 25% decrease*
        - 50ppm: 19% decrease
        - 75ppm: 13% decrease
    - Hair development (number of days):
        - 20ppm: 11% decrease*
        - 50ppm: 15% decrease*
        - 75ppm: 2% increase
    - Incisor eruption: No significant change
    - Eyelid opening: No significant change
    - Surface righting: No significant change

- Parturition time:
    - 20ppm: 6% longer
    - 50ppm: 19% longer
    - 75ppm: 42% longer

- Pup body weights: No significant change

- Pup organ weights:
    - Liver weight: No significant change

  - Brain weight: No significant change

- Liver protein levels: No significant change in either pups or dams

- Liver DNA levels: No significant change in either pups or dams

- Liver cholesterol levels:
  - 20ppm:
    - Pups:
      - Day 1: 21% decrease
      - Day 7: 1% increase
      - Day 14: 3% increase
      - Day 21: 10 % decrease*
      - Dams: 1% decrease
  - 50ppm:
    - Pups:
      - Day 1: 16% decrease
      - Day 7: 2% increase
      - Day 14: 10% increase*
      - Day 21: 0.4% decrease
      - Dams: 3% decrease
  - 75ppm:
    - Pups:
      - Day 1: 1% increase
      - Day 7: 4% increase
      - Day 14: 7% increase
      - Day 21: 3% decrease
      - Dams: 6% increase*

- Brain protein levels: No significant change

- Brain DNA levels: No significant change

- Brain cholesterol levels:
  - 20ppm: No significant change
  - 50ppm:
    - Pups:
      - Day 1: 14% decrease
      - Day 7: 1% increase
      - Day 14: 8% increase*
      - Day 21: 1% decrease
      - Dams: 4% decrease

- 75ppm:
  - Pups:
    - Day 1: 14% increase
    - Day 7: 2% increase
    - Day 14: 8% increase*
    - Day 21: 0.3% decrease
  - Dams: 7% increase

*Statistically significant

Author's Conclusions:

- *"A decrease in maternal food intake was noted at the time of initial exposure of the dam to 50 and 75ppm H_2S, followed by recovery of appetite after several days…did not appear to cause a change in weight gain."*
- *"Exposure…did not cause a significant change from matched controls in pup or maternal brain or liver weight, length of gestation, litter size, pup birth weight, male to female ratio or viability."*
- *"Maternal levels of cholesterol were elevated in liver and possibly in brain samples after the dam had been exposed to 75ppm H_2S for approximately 6 weeks although there was no consistent change in pup cholesterol levels.*
- *"…adult rats appear to be more susceptible to H_2S insult possibly because of slowed metabolism or protein synthesis."*
- *"The acceleration of hair development and ear detachment observed at lower concentration of H_2S…could indicate an alteration of development caused by the interaction of H_2S or a metabolic product of H_2S with a growth signal…the greater effect…at low concentrations suggests that H_2S could have a biphasic toxicity with an acute response at high concentration and an alternate response mechanism with chronic low concentration exposure."*
- *"The mean parturition time was extended at all levels of exposure and appeared to be dose dependent."*
- *"…many of the dams having a normal length of labor, but the remaining experimental animals, six of eighteen observed, and a single control dam had extended and difficult deliveries."*
- *"Analysis of organ weight gain, protein, DNA and cholesterol accumulation as an index of growth and development indicated that the rat pups exhibited a normal growth pattern similar to that previously documented. None of the values were significantly different from the matched controls."*
- *"In summary, low levels of H_2S during gestation and neonatal development to day 21 PP alters observed ear detachment and hair development in pups and H_2S at all levels monitored extended parturition time and possibly elevated cholesterol content of the maternal liver and brain."*

Review – Assessment:

A. Test animals:	- It was unclear how may test animals were used in total. It was stated that *"twenty gravid females were placed in two matched groups"* but it was unclear whether this indicated that 20 animals were divided into 10 treated and 10 control rats or alternatively that 20 animals were allocated to each of these groups. Based on the information supplied, it appeared most likely that 10 gravid females were assigned to each group. This number is limited in relation to the number of dams (n=20) recommended by most testing guidelines for developmental toxicity. - The number of pups examined was not clearly specified, and evidently varied by group and by the endpoint being monitored. The methods section and litter size data would suggest that for any given experiment a total of approximately 72 pups per exposure concentration should have been available for examination (*i.e.*, groups were reportedly culled to 6 dams with a maximum of 12 pups per litter on Day 1 post-partum). However, the number of pups listed as being examined for the various endpoints monitored could not be readily matched against this total. - The number of litters examined (*i.e.*, as few as 6 per exposure concentration) was limited. Guidelines for developmental toxicity testing generally recommend examination of 20 litters per treatment group. + The strain and specie of the test animal were reported. - The pre-test health and source of the dams were not specified.
B. Exposure conditions:	+ The source of the H_2S gas was reported. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. +/- It is unclear whether animals were placed in the exposure chamber before or after the equilibration period. However, equilibrium of gas concentration was obtained rapidly (*i.e.*, within 10 min of gas introduction) + The total animal displacement in exposure chambers reportedly did not exceed 5% of the total volume of the chamber, consistent with guideline recommendations. + The chamber was designed to permit continuous observation of the animals. +/- H_2S concentrations, airflow (20 changes per hour), oxygen content (20-21%), temperature, pressure, humidity and photoperiod were monitored throughout exposure, but the results of most of these analyses were not reported. Accordingly, it was not possible to verify that the chamber conditions met guideline recommendations, nor to confirm that the target concentrations of H_2S were achieved. +/- Concentrations of H_2S were measured at the animal level, but these levels were not reported. + Previous testing verified that H_2S was homogeneously distributed throughout the chamber.
C. Housing/Feeding:	- Other than specification of the photoperiod, details concerning environmental conditions within the animal room were not supplied. - It is unclear as to whether the dams and/or pups were housed continuously in the exposure chamber or introduced daily into the chamber during the 7-hour exposure period only. +/- The material from which cages was constructed was noted but information pertaining to cage dimensions was lacking. - Bedding and nesting materials were not specified. + Animals were allowed to eat and drink *ad libitum* except during the exposure period, consistent with guideline recommendations. - The source and type of feed was not noted. - Details concerning the water supply were not provided.
D. Equipment:	+ The exposure device was adequately described (*i.e.*, 90L acrylic dome-shaped inhalation chamber). More complete details were found in a companion paper (Laskin and Drew, 1970). +/- The equipment used to measure gas concentration (*i.e.*, pre-calibrated GFG model GMA-monitoring system) and airflow rate

	(*i.e.*, orifice plate) were reported, but the manner by which humidity, temperature, and oxygen levels were determined was not outlined.
E. Procedural:	+/- Animals were acclimatized, but the period of acclimatization was half of that recommended by testing guidelines. - No description of pre-test conditions was provided. +/- Pups were randomly chosen for examination, but whether dams were randomly assigned to exposure groups was not noted. Details concerning the methods of randomization (*e.g.*, table of random numbers, computer generated, etc.) were not supplied. It is of some interest that the body weights of the dams shortly before initiation of treatment exhibited a downward trend across the treatment groups, suggesting that if the dams were randomly allocated to groups, there was no adjustment based on body weight considerations. - On Day 1 post-partum, groups were culled to 6 dams with a maximum of 12 pups per litter. This culling practice represents a departure from guideline recommendations. Typically, dams are not culled, but rather are maintained along with their litters to ensure adequate and representative numbers of dams, litters and pups are available for examination. Culling is generally performed on a litter basis on Day 4 post-partum (*i.e.*, litters are culled to a maximum of 8 pups) and is intended to reduce the level of stress of the nursing mother and to reduce inter-litter variability. By culling dams, information was lost. + Controls were *"maintained under identical conditions"* but without H_2S exposure. - The reason why two separate 20ppm exposures were carried out was not outlined. - Unclear whether technicians and handlers were blinded to exposure conditions. + Food and water consumption were monitored throughout gestation and maternal weights were observed until the dam was sacrificed. + The male to female ratio and viability of pups at birth and throughout the neonatal exposure period were monitored. + The physical development of exposed and matched control pups were compared (e.g., pinna detachment, incisor eruption, eyelid opening and weight gain) + An assessment of motor development was conducted. + Serum, liver and brain tissue samples were collected. - No indication that study was performed under Good Laboratory Practice (GLP) conditions. +/- Three treatment levels and a corresponding control group were tested, thereby permitting investigation of a dose-response relationship; however, it would appear that treatments were performed in series at separate times, thereby confounding interpretation of findings. - Clinical observations of dams and pups were not reported.
F. Data collection:	- Individual data were not provided for each test animal, thereby, limiting the independent assessment of the findings. + Control data were combined only *"after it was established that each set of control data possessed a similar mean value"*. - The number of animals analyzed for each parameter varied. - If a standard scale was used to assess developmental changes, this was not specified.
G. Data analysis:	+ *"Nonparametric data were analyzed using the Mann-Whitney U-test for significance"* + *"...parametric data were compared using analysis of variance and Duncan's Multiple Range Test or Student-Newman-Keuls procedure."* - The statistical significance of some parameters (*e.g.*, parturition time) was not provided. - Intra-litter comparisons were not conducted. - Confidence intervals were not provided. - SEM was notably large for multiple measurements.
H. Interpretations:	- Potential confounding variables (*e.g.*, selection bias with respect to dams, litters, pups; failure to use the litter as the basis of comparison) were not addressed by either the study design or statistical analyses.

	- Conclusions were largely based upon hypothesis, without the support of empirical data. - According to table 3 (p. 395), pinna detachment and hair development were generally accelerated in the exposed groups. This assessment is supported by the statement on p. 399 that " *The acceleration of hair development and ear detachment observed at lower concentrations of H_2S.....* " . Yet on p. 395, the authors' state that "*Pinna detachment and hair development were delayed in all exposed animals with a longer delay at the lower concentrations.*" - Despite the authors' conclusions, the changes in pinna detachment and hair development were not dose-responsive. - The dystocia reportedly observed among the treated groups was inconsistent between dams and showed a very high degree of variability not only between dams, but also between experiments (i.e., parturition time varied by up to 50% even among control groups studied at different times).

Review - Summary:

Discussion of findings:

Exposure of gravid rat dams to 20, 50 or 75 ppm of H_2S from Day 6 of gestation to Day 21 post-partum reportedly caused dystocia at all exposure concentrations as well as elevated maternal liver cholesterol content at 75 ppm. A transient depression in feed intake was noted for the treated dams during the first few days following initiation of treatment; however, normal feed intake was restored within one week. The reduced feed intake likely reflected maternal stress. Body weights of the treated dams were unaffected. Pups exposed *in utero* and postnatally until Day 21 post-partum reportedly showed developmental deficits, characterized by accelerated pinna detachment and accelerated hair development. Other maternal parameters were unaffected by treatment, including maternal body weight and weight gain during gestation, maternal liver and brain weights, liver and brain protein content, liver and brain DNA content, and brain cholesterol levels. Routine reproductive parameters also were unaffected by treatment, including length of gestation, litter size, pup viability and pup sex ratio. In addition, all remaining pup parameters were unaffected by treatment, including body weights, brain weights, liver weights, liver and brain protein content, liver and brain DNA content, liver and brain cholesterol levels, and other developmental indices (*i.e.,* eyelid opening and surface righting).

Interpretation of the significance of the observed changes should take into consideration the following:

- The reported dystocia affecting the treated dams appeared to be based on the observation of limited numbers of animals. The parturition times summarized in the report accounted for only 5 to 6 dams per treatment group. (Regrettably, the total number of dams available for observation was not reported). Guidelines for developmental toxicity studies generally recommend that reproductive parameters be followed in 20 dams per treatment level.
- The reported dystocia was "*animal dependent, with many of the dams having a normal length of labour*". Unfortunately, the incidence of dystocia could not be calculated since the total number of dams on test was not reported. Without clear indication of the frequency of the extended labour and the total number of dams affected, it is very difficult to assign a level of significance to the findings.
- The summary data on parturition times contained in the report showed large standard errors surrounding the mean values, indicating a high degree of inter-animal variability and supporting the statement that the dystocia was "*animal dependent*".
- Parturition times were increased in a dose-related manner, with a reported increase of 10, 20 and 42 percent over matched controls at 20, 50 and 75 ppm, respectively. However, parturition times also varied across the series of experiments, as evidenced by differences in the times reported for each of the control groups. These differences ranged from 15 percent (*i.e.,* control for 20 ppm exposure vs. control for 75 ppm exposure) to more than 50 percent (*i.e.*, control for 50 ppm exposure vs. control for 75 ppm exposure).
- No evidence was provided to indicate that the differences in parturition times between the treated groups and the corresponding controls achieved statistical significance (presumably owing to the large standard errors).
- The reported dystocia evidently did not adversely affect the health of the dams; however, "*a few*" fetuses reportedly died due to asphyxiation related to the extended delivery time. Regrettably, the exact number of fetuses that died was not reported, nor could the corresponding incidence data be calculated. Moreover, the pups deaths were not allocated to treatment groups, and thus, the dose-responsiveness of the effect could not be

determined.

- The reported increase in maternal liver cholesterol content was not dose-responsive. The change applied only to the dams exposed to 75 ppm. The cholesterol levels measured in the dams exposed to 20 and 50 ppm were *decreased* relative to the matched controls.
- Although the increase in maternal liver cholesterol content observed among the dams exposed to 75 ppm achieved statistical significance, the absolute increase was only 6 percent relative to the corresponding control group.
- The reported changes in pinna detachment and hair development observed among the treated pups were not dose-responsive. The reduced time for pinna detachment was most pronounced at 20 ppm and least pronounced at 75 ppm. Similarly, the time to hair development was reduced in the pups exposed to 20 and 50 ppm, but marginally *increased* in the pups exposed to 75 ppm. In both instances, the reported changes in development did not uniformly achieve statistical significance, but rather, the differences from the corresponding control values were statistically significant only for the pups exposed to 20 ppm (pinna detachment and hair development) and 50 ppm (hair development only).

The above considerations undermine the level of significance that can be assigned to the reported changes in maternal health, reproductive parameters and neonatal development, especially those changes for which dose-responsiveness was not evident. The difficulty surrounding the interpretation of the significance of the findings is further enhanced by the following:

- Exposures evidently were performed through a series of experiments, each involving a separate control group. This practice renders comparison of findings across treatment groups more difficult than the conventional treatment protocols recommended by testing guidelines, in which exposures are performed concurrently in order to minimize influences other than treatment on experimental outcomes.
- The culling of groups to 6 dams at the time of parturition, without justification and with no description of selection methods, resulted in: i) the introduction of possible selection bias; and, ii) loss of confidence in the findings owing to the restricted numbers of litters from which pups were obtained for examination. Testing guidelines for developmental toxicity generally recommend use of 20 dams per treatment group and that all litters be followed post-parturition.
- Pup numbers were not consistently reported. According to the Methods section of the report, groups were culled to 6 dams with a maximum of 12 pups per litter. Since average litter size across all groups ranged from 13 to 14, the number of pups available for examination should have approached 288, 144, 72 and 72 for the control, 20 ppm, 50 ppm and 75 ppm groups, respectively. However, these pup numbers could not be easily matched to the summary data provided in the report for the various growth and developmental endpoints monitored, even after accounting for interim sacrifices of the pups performed at Days 1, 7, 14 and 21 post-partum for tissue analyses. For example, the report states that at each interim sacrifice time, *"all remaining pups were weighed"*; however, the body weight data recorded for Day 7 list the pup numbers as 60, 40, 23, and 17 for the control, 20 ppm, 50 ppm, and 75 ppm groups, respectively. These numbers appear to be too low based on the initial number of pups available for observation. Moreover, the pups numbers *increased* across all groups on Days 14 and 21 despite sequential interim sacrifices. It was virtually impossible to account for the pup numbers listed in the report, thereby adding confusion to the interpretation of the study findings.
- The findings relating to neonatal growth and development were based only on the pup as the unit of comparison. There was no attempt to use the litter as the unit of comparison. Guidelines for developmental toxicity testing generally recommend the use of both units for comparison.

Any assessment of the relevance of the study findings to the human condition should consider the nature and duration of the exposure regimen, involving daily exposures to H_2S from Day 6 of gestation to Day 21 post-partum. The equivalent human exposure time would extend over several months

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to, undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of only limited numbers of dams and litters, especially at the 50 ppm and 75 ppm exposure levels (*i.e.*, 6 per treatment level).
- Failure to conduct exposures concurrently.
- Lack of detail concerning litter selection.

- Failure to report clinical signs other than those related to delivery.
- Failure to use the litter as the unit of comparison.
- Failure to consistently report pup numbers.
- General lack of detail concerning animal housing and husbandry.

Review – Confidence index ranking:

Moderate to low

Document Review

Author:	Bhambhani, Y; Burnham, R; Syndmiller, G; MacLean, I; Martin, T	**Internal ID:**	118
Title:	Effects of 5ppm hydrogen sulfide inhalation on biochemical properties of skeletal muscle in exercising men and women.		
Year:	1996		
Abstract:	*"This study compared the acute effects of 5 ppm hydrogen sulfide (H_2S) inhalation (50% of its occupational exposure limit) on the biochemical properties of skeletal muscle in exercising men and women. Twenty-five healthy volunteers, 13 men and 12 women, completed two 30-minute submaximal tests at 50% of their predetermined maximal aerobic power (VO_{2max}) while breathing 0 ppm (control) or 5 ppm H_2S from a specially designed flow system in a single-blind manner. Immediately after exercise, biopsies were obtained from the vastus lateralis muscle under local anesthesia. They were subsequently analyzed for concentrations of the following markers of anaerobic and aerobic metabolism; lactate (La), lactate dehydrogenase (LDH), citrate synthase (CS), and cytochrome oxidase (CytOx). Repeated measures analysis of variance indicated that in men, the CS concentration decreased significantly (p-0.006) as a result of H_2S exposure. There was also a tendency for their La and LDH concentrations to increase and CytOx concentration to decrease in the presence of H_2S, but these changes were not significant ($p>0.05$). In women no significant changes were observed in any of these biochemical properties. These results suggest that (1) exposure to H_2S at 50% of its OEL might inhibit aerobic metabolism during exercise in healthy men, thereby increasing their dependency on anaerobic metabolism; and (2) there could be a significant gender difference in the acute response to sub-lethal exposures of H_2S."*		
Objective:	To investigate the effects of oral inhalation of 5ppm H_2S on the biochemical properties of skeletal muscle in exercising men and women. (A series of enzymatic markers of aerobic and anaerobic metabolism were monitored in men and women performing submaximal exercise).		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0 or 5ppm (by mouthpiece)	Single/ 30 minutes. Exposures were performed in replicate, once a week for three weeks.	Both	Men: 24.7 +/-4.6 yr Women: 22.0 +/-2.1 yr	25. (13 men; 12 women)	Healthy	5ppm for women	5ppm for men

Observations:

Objective responses:

- Oxygen uptake ($V0_2$) and expired ventilation volume (V_E) were significantly lower in women than in men. Marginal numerical increase (less than 5%) and marginal numerical decrease (less than 5%) in VO_2 in men and women, respectively, following H_2S exposure.

- Men:
  - VO_2: Less than 5% increase
  - V_E: Increase
  - Muscle lactate (La) concentration: 24% increase
  - Lactate dehydrogenase (LDH) concentration: 6% increase
  - Citrate sythetase (CS) concentration: 19% decrease*
  - Cytochrome oxidase (CytOx) concentration: 9% decrease
  - Only the change in CS activity achieved stastistical significance

- Women:
  - $V0_2$: Less than 5% decrease

- V_E: Increase
- Muscle La concentration: No change
- LDH concentration: No change
- CS concentration: 19% decrease
- CytOx concentration: 23% increase

Subjective responses:

- None reported. No indication of undue fatigue, headache, nausea or any other symptoms during or following exposure.

*Statistically significant

Author's Conclusions:

- *"The only significant finding .was the decrease in CS concentration in men exposed to 5ppm H_2S. No significant alterations were observed in the other markers of anaerobic and aerobic metabolism, namely, concentrations of La, LDH, and CytOx in both genders".*
- *"..it was hypothesized that inhalation of 5ppm H_2S during moderate exercise would alter the biochemical profile of skeletal muscle by increasing the relative contribution of the anaerobic component with a concomitant decrease in the aerobic component. The overall findings suggested that this seemed to be the trend in men but not in women."*
- *"In conclusion, inhalation of 5 ppm H_2S during submaximal exercise decreases the concentration of CS in the skeletal muscle of men but not in women. No significant changes in other markers of anaerobic and aerobic metabolism, namely concentrations of La, LDH, and CytOx were observed as a result of this exposure in both genders".*
- *"There seemed to be tendency for the metabolic profile to shift from aerobic to anaerobic conditions in men, which is consistent with observations at high concentrations in the animal species".*
- *"These results suggest that (1) exposure to H_2S at 50% of its OEL might inhibit aerobic metabolism during exercise in healthy men, thereby increasing their dependency on anaerobic metabolism; and (2) there could be a significant gender difference in the acute response to sub-OEL exposures to H_2S".*

Review – Assessment:

A. Subjects:	+/- 25 subjects were enrolled in the study, but certain analyses were performed only on a subset of this 25 (presumably due to limited size of tissue biopsy samples). Since the results were segregated by sex, the number of subjects was somewhat limited. + Subject profiles (gender, age, weight, hemoglobin levels) were adequatety described. + Clear exclusion criteria were utilized for the selection of volunteers. 'High risk' individuals, as defined by the Illinois Institute of Environmental Quality (1964), and those previously chronically exposed were excluded. - Individuals on average had a higher $V0_{2max}$ than the average population. Subjects were more aerobically fit than the general population. + The study was conducted on both males and females. + Informed consent was given.
B. Exposure conditions:	+ The concentration of H_2S was continuously monitored and analytically confirmed. + The precise duration of exposure was recorded. +/- A mouthpiece was used to administer H_2S. This exposure method reduces detection bias but omits potential physiological responses triggered by smell. + All exposure conditions (*e.g.*, airflow, humidity and concentration) with the exception of temperature were monitored.
C. Equipment:	+ A full description of the exposure device and monitoring equipment was provided to permit reproducibility.
D. Procedural:	+ Subjects were exposed randomly.

	+ Subjects and technicians conducting the biochemical analysis were blinded to exposure conditions. + Subjects served as their own controls, thus limiting confounding variables.
E. Data collection:	+/- Analysis of LDH was conducted on only six samples per gender. Lactate levels were determined in only seven biopsies in men and six biopsies in women (presumably due to limited tissue sample size). CS and CytOx assays were performed on tissue samples from all subjects. - Dilutions required to perform enzymatic assays might have lead to an underestimation of effect of H_2S upon the measured enzymes + Duplicate measurements of lactate dehydrogenase, citrate synthetase and cytochrome oxidase levels were conducted. + Observations were objective.
F. Data analysis:	+ Statistical analysis was judged to be appropriate. - Confidence intervals were not provided. - Individual data were not provided limiting the independent assessment of results.
G. Interpretation:	- Exposure was conducted during sub-maximal exercise. When extrapolating findings of this study to resting exposure, this should be considered. - No correction was made for the lower ventilation rate, and hence, exposure of women. - Several findings were inconsistent between subjects and between sexes. For example, in men, 54% showed a decrease, 23% showed an increase and 23% showed no change in CytOx activity following exposure. Similarly, in women, 58% showed a decrease, 17% showed an increase and 25% showed no change in CytOx activity. On balance, CytOx activity was numerically reduced in men, but increased in women. In addition, muscle lactate level was increased in men, but decreased in women following exposure.

Review - Summary:

Discussion of findings:

Bhambhani *et al* hypothesized that inhalation of 5ppm H_2S during a period of thirty minutes of moderate exercise would shift the biochemical profile of skeletal muscle in healthy young men and women. However, the expected suppression of the aerobic biomakers (*e.g.*, citrate synthetase, cytochrome oxidase) and concomitant increase in anaerobic markers (*e.g.*, lactate and lactate dehydrogenase) was only observed in the exposed males. In women, markers of aerobic metabolism increased, whereas indicators of anaerobic metabolism remained unchanged. None of the observed changes were significant except the decrease in citrate sythetase levels in men. The authors attributed the absence of significant changes in lactate levels to the higher than average $V0_{2max}$ of subjects, quoting previous studies which established an inverse relationship between blood lactate and $V0_{2max}$.

The authors postulated that the biochemical shift would be more pronouced in women than men due to the typically lower blood volumes and hemoglobin content of females; however, as noted above, this was not observed. As noted by the author, *"the only significant finding..was the decrease in citrate synthetase levels in men"*, a change which resulted in no observable health effects. Any shift toward anaerobic metabolism noted among the male subjeects was modest, as evidenced by the lack of significant changes in the majority of biochemical markers. In women, no indication of a shift toward anaerobic metabolism was evident. It was stated that "*it is unclear from the present findings whether there is a gender difference in the response to H_2S*" . These findings suggest, according to the authors, that exposure to 5 ppm H_2S, even under conditions of moderate exercise, is fully tolerable.

Interpretation of the significance of the study findings and their relevance to the general population should consider the following:

- Subjects were performing exercise to 50% of their maximum capability during exposure. Accordingly, the inhaled dose of H_2S was relatively high, and certainly greater than that which might be received under resting conditions.

- Participants were young and healthy.
- Exposure time was limited (*i.e.*, 30 minutes).
- A mouthpiece was used to administer H_2S. Hence, psychological implications of the odor of H_2S and the irritant capacity of the gas on the mucous membranes of the nose and eyes were not assessed.
- The changes in biochemical parameters recorded for the male subjects were *suggestive* of a possible shift from aerobic to anaerobic metabolism in response to H_2S exposure (*i.e.*, marginal increase in muscle lactate concentration and LDH activity, and marginal decrease in cytochrome oxidace and citrate synthetase activities). However, the strength of the association is necessarily weakened by the lack of consistency of response between subjects, the relatively modest nature of the changes, and the general lack of statistically significant findings.A similar shift toward anaerobic metabolism was *not* evident among the female subjects.
- The general lack of response among the female subjects compared to the males could possibly be related to a lower amount of inhaled H_2S.
- The reportedly observed shift toward anaerobic metabolism in the males is not unique to H_2S exposure. Such shifts occur regularly in response to increased physical activity (*e.g.*, exercise), and as part of normal diurnal rhythms (*e.g.*, following overnight fasting).
- No clinical correlates of the observed shifts in biochemical parameters were determined in either sex..No clinical symptoms were recored among the test subjects either during exposure or 24 hours post-exposure. Thus, it would appear that any change in metabolic profile in response to exposure occurred only at the cellular level, and was not great enough to be expressed clinically.

Confidenced index:

No significant omissions in study design, conduct or reporting were noted, but the external validity of the study could have been improved by:

- Conducting trials under resting conditions as well as under conditions of moderate exercise.
- Increasing the number of test subjects. (Note that the authors ascribed the lack of statistically significant changes in certain of the variables to small sample size).
- Participation of individuals more representative of the general population.
- Calculating the dosage received by treated individuals, with consideration to subject specific ventilation rates.
- Exposure of graded exposure concentrations to permit assessment of dose-responsiveness.
- Presentation of individual subject data to permit more thorough interpretation of the findings, especially since changes in the measured parameters evidently varied considerably between individuals.

Review – Confidence index ranking:

High

Document Review

Author:	Bhambhani, Y; Singh, M	**Internal ID:**	119
Title:	Physiological effects of hydrogen sulfide inhalation during exercise in healthy men.		
Year:	1991		
Abstract:	*"Occupational exposure to hydrogen sulfide (H_2S) is prevalent in a variety of industries. H_2S when inhaled 1) is oxidized into a sulfate or a thiosulfate by oxygen bound to hemoglobin and 2) suppresses aerobic metabolism by inhibiting cytochrome oxidase (c and aa_3) activity in the electron transport chain. The purpose of this study was to examine the acute effects of oral inhalation of H_2S on the physiological responses during graded cycle exercise performed to exhaustion in healthy male subjects. Sixteen volunteers were randomly exposed to 0(control), 0.5, 2.0, and 5.0 ppm H_2S on four separate occasions. Compared with the control values, the results indicated that the heart rate and expired ventilation were unaffected as a result of the H_2S exposures during submaximal and maximal exercise. The oxygen uptake had a tendency to increase, whereas carbon dioxide output had a tendency to decrease as a result of the H_2S exposures, but only the 5.0 ppm exposure resulted in a significantly higher maximum oxygen uptake. Blood lactate concentrations increased significantly during submaximal and maximal exercise as a result of the 5.0 ppm exposure. Despite these large increases in lactate concentration, the maximal power output of the subjects was not significantly altered as a result of the 5.0 ppm H_2S exposure. It was concluded that healthy young male subjects could safely exercise at their maximum metabolic rates while breathing 5.0 ppm H_2S without experiencing a significant reduction in their maximum physical work capacity during short-term incremental exercise."*		
Objective:	To examine the acute effects of oral inhalation of H_2S on the physiological responses during graded cycle exercise, performed to exhaustion, in healthy male subjects. The study investigators wished to test several hypotheses surrounding the metabolic responses induced by exposure to hydrogen sulphide through examination of the interrelationships between oxygen uptake, carbon dioxide output, blood lactate concentration, and physical work capacity.		

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0ppm; 0.5ppm; 2.0ppm; 5.0ppm (by mouthpiece)	Single exposure lasting a minimum of 16 minutes. Four replicates were performed, once every two weeks, while breathing one of the 4 exposure concentrations in random order.	Male	25.2 +/-5.5 yrs.	16	Normal	2.0 ppm (?)	5.0ppm

Observations:

Objective responses:

- Maximal power output (MPO): No significant change

- Pulmonary effects:
  - 0_2 uptake ($V0_2$): Tendency to rise at all three exercise intenities (*i.e.*, ventilatory threshold 1, ventilatory threshold 2 and maximal power ouput) , but a significant change was only observed at 5ppm during MPO*. Change at 5 ppm ranged from 6 to 10%.
  - $C0_2$ output:
    - No significant change.
    -Tendency to exhibit an inverse relationship with the concentration of H_2S at MPO. (Change was less than 6 %).
  - Ventilation rate: No significant change.
  - Respiratory exchange ratio (RER) (calculated):
    - 2ppm: Reduction at MPO*

- 5ppm: Reduction at "*ventilatory threshold 1*" (VT1) and MPO*
  - Tendency to exhibit an inverse relationship with the concentration of H_2S.
- Expired ventilation volume: No significant change.

- Metabolic effects:
  - Lactate concentration in venous blood:
    - 5ppm: Increase at all exercise levels.* (Change in blood lactate concentration ranged from 44 to 65%).
    - Tendency to rise as the concentration of H_2S increased.
  - $V0_{2max}$ appeared to be inversely related to lactate concentration.

- Cardiovascular effects:
  - Heart rate: No significant change.

Subjective responses:

- None reported

*Statistically significant

Author's Conclusions:

- *"...healthy young males were able to safely exercise at their maximum metabolic rates while orally breathing 5.0ppm H_2S without experiencing a significant reduction in their maximum physical work capacity during short-term incremental exercise."*
- *"The results indicated that the primary physiological response of inhalation of 5.0 ppm H_2S was to increase the blood lactate concentration compared with control conditions."*
- *"The primary physiological response under these conditions seemed to be an increased lactate accumulation during submaximal and maximal exercise, which could be due to an enhanced intramuscular lactate production as well as a decrease in its rate of removal from the blood by the nonexercising tissues. This increase in blood lactate concentration, however, did not significantly reduce the maximum physical work capacity of the subjects during short-term incremental exercise."*
- *"The elevated blood lactate response observed under the influence of H_2S seemed to be dependent on the following three factors: 1) concentration of H_2S, 2) intensity of exercise, and 3) $V0_{2max}$ of the subjects."*
- *"The minimum concentration of H_2S required to elicit the elevated blood lactate response was between 2.0 and 5.0ppm."*
- *"The results of this study suggested that the VO_{2max} of the subjects did have some influence on the accumulation of lactate due to H_2S exposure, because a significant negative correlation was observed between the VO_{2max} of the subjects and the difference in lactate concentrations between the 5 ppm and control exposures at the MPO. Physiologically, this might be because individuals with higher aerobic powers are known to have larger blood and hemoglobin volumes and greater cytochrome oxidase activities than those with lower aerobic powers, which could minimize the toxic effects of H_2S during exercise".*

Review – Assessment:

A. Subjects:	+ The number of subjects was adequate. +/- Subject profiles (gender, age and weight) were adequately described. + Clear exclusion criteria were utilized in the selection of volunteers. 'High risk' individuals, as defined by the Illinois Institute of Environmental Quality (1964), and those previously exposed were excluded. + Subjects of normal physical activity patterns were selected, thereby, improving the external validity of this study (*i.e.*, the study subjects were not involved in systematic endurance training programs).

	- Only male subjects were included. Thus, the extrapolation of this study's findings should be limited to this gender. + Informed consent was given.
B. Exposure conditions:	+ The concentrations of the gas to which subjects were exposed was monitored and analytically confirmed. - The precise duration of exposure was not recorded. The period of time required for subjects to reach maximal power output determined the exposure duration. The length of this period was not specified, but rather it varied by subject. +/- A mouthpiece was used to administer H_2S. This exposure method reduces detection bias but omits potential physiological responses triggered by smell. + All exposure conditions (*e.g.*, airflow, humidity and concentration) except temperature were monitored.
C. Equipment:	+ A full description of the exposure device and monitoring equipment was provided.
D. Procedural:	+ Subjects were exposed randomly. + Both the subjects and technicians involved in monitoring the physiological parameters and conducting the laboratory analyses were blinded to the exposure concentration (including the control level). + Subjects served as their own controls, thus limiting confounding variables. - No follow-up exams were conducted.
E. Data collection:	+ The plasma component of branchial vein blood samples were analyzed. Venous blood contains higher levels of lactate than that of arterial blood, hence this analysis is more sensitive. + All symptoms recorded were objective. - The $V0_{2max}$ was calculated utilizing the equation: (maximal cardiac output) x (maximal arteriovenous oxygen content difference). If the investigators measured arterial $P0_2$ it is unclear when this was conducted. - Blood lactate levels are affected by the duration of time that has elapsed since the subject's last meal. Consequently, this period of time should have been standardized in the experimental procedure.
F. Data analysis:	+ All statistical analysis was conducted using the SPSSx computer program. - Confidence intervals were not provided. - Individual data were not provided to permit independent assessment of the results.
G. Interpretations:	- Exposure was conducted during submaximal and maximal exercise. - Investigators did not compare the physiological parameters of a normal person at rest with the measured values. - It is unclear what type of everyday activities correspond to the first and second ventilatory thresholds.

Review - Summary:

Discussion of findings:

A significant decrease in the respiratory exchange ratio (RER) was observed following exposure to 2ppm and 5ppm H_2S during maximal and submaximal exercise respectively. The 10% decrease noted at 2ppm represented the only change achieving statistical significance at this exposure concentration. Significant increases in oxygen uptake and blood lactate were noted after 5ppm H_2S exposure, at all three levels of exercise intensity tested. The authors concluded that the degree of lactate elevation was proportional to the concentration of H_2S and exercise intensity, but inversely related to the $V0_{2max}$ of the exposed individual.
Overall no significant changes in ventilation rate, expired ventilation volume or cardiovascular parameters were detected. Carbon dioxide and oxygen uptake tended to be inversely related to the concentration of H_2S but the observed changes were not significant. The primary focus of this study was to test the following hypotheses concerning the effect of H_2S upon oxygen transport to tissues:

a) Oxygen detoxification of H_2S decreases the total oxygen transport to tissues, thereby increasing dependency on anaerobic metabolism. The resulting increase in blood and muscle lactate levels would then likely be reflected in premature muscular fatigue.

b) Oxygen detoxification of H_2S results in an increase in total oxygen uptake.

Hypothesis (a) was supported by the findings of this study, except no significant change in maximal power output was observed.

Interpretation of the significance of the study findings should consider the following:

- No clinical symptoms were reported by any of the subjects during or following exposure. There were no complaints of headache, nausea, dizziness, sore throat *etc.*
- Subjects were performing exercise to exhaustion during the trial.
- The study was performed using male subjects only. Effects on the female sex were not examined.
- A mouthpiece was used to administer H_2S. Hence psychological implications of the odor of H_2S as well as responses related to the potential irritant effects of the gas on the mucous membranes of the nose and eyes were not assessed.
- The effects are consistent with the widely-accepted mechanism of action of H_2S (*i.e.*, inhibition of cytochrome oxidase), but they are not specific to H_2S exposure and occur to varying degrees during normal activity and as part of normal diurnal rhythms. The primary physiological response observed following 5ppm H_2S exposure was an increase in blood lactate concentration (up to 65%). This is a minimal increase when compared to the 450% increase in blood lactate levels simply attributed to rising exercise intensity. Further, this biochemical change resulted in no detectable clinical symptoms.
- Effects were monitored at three different intensities of exercise: ventilatory threshold 1, ventilatory threshold 2, and maximal power output. As the author provided no indication of the types of physical activities that correspond to these exertion levels, it is difficult to provide perspective on the everyday relevance of the experimental findings.
- RER represents a calculated versus a measured value.

Confidence index:

No significant omissions in study design, conduct or reporting were noted, but the external validity of the study could have been improved by:

- Conducting trials at resting conditions.
- Inclusion of both genders, to expand the external validity of the study's findings.
- Calculating the dosage received by treated individuals, with consideration to subject specific ventilatory rates.
- Addition of follow-up exams to assess the reversibility of changes.

Review – Confidence index ranking:

High

Document Review

Author:	Bhambhani, Y; Burnham, R; Syndmiller, G; MacLean, I; Martin, T	**Internal ID:**	120
Title:	Comparative physiological responses of exercising men and women to 5ppm hydrogen sulfide exposure		
Year:	1994		
Abstract:	*"A study compared the effects of inhalation of 5 ppm hydrogen sulfide (50% of its occupational exposure limit) on the physiological and hematological responses of healthy men and women during exercise. Subjects were 13 men (mean ± SD for age, height, and weight = 24.7 ± 4.6 yr, 173 ± 6.6 cm, 73.1 ± 8.1 kg, respectively) and 12 women (mean ± SD for age, height, and weight = 22.0 ± 2.1 yr, 165 ± 8.2 cm, 63.4 ± 8.6 kg, respectively). Subjects completed two 30-minute exercise tests on a cycle ergometer at 50% of their predetermined maximal aerobic power while breathing medical air or 5 ppm H_2S from a specially designed flow system. The results indicated that there were no significant differences between the two exposures for the metabolic (oxygen uptake, carbon dioxide productions, respiratory exchange ratio), cardiovascular (heart rate, blood pressure, rate pressure product), arterial blood (oxygen and carbon dioxide tensions, pH), and perceptual (rating of perceived exertion) responses in either six. None of the subjects reported any adverse health effects subsequent to the H_2S exposure. These results suggest that healthy men and women can safely perform moderate intensity work in environments contaminated with 5 ppm H_2S."*		
Objective:	To compare the acute effects of 5ppm H_2S inhalation on selected physiological and hematological parameters during 30 min of submaximal exercise in healthy men and women. A specific hypothesis tested was that inhalation of 5 ppm H_2S would induce a significantly greater increase in lactate concentration in women than in men because of the lower amount of oxygen that women would have available for detoxification purposes.		

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0 and 5ppm (by mouthpiece)	Single exposure/ lasting 30 minutes. Three replicate exposures were performed on each subject over a three-week period.	Both	Adult	25 (13 men; 12 women)	Healthy	5ppm	NA

Observations:

Objective responses:

- Pulmonary effects:
  - O_2 uptake ($V0_2$): No significant change
    - -Males: Modest numerical increase (less than 5% change)
    - -Females: Modest numerical decrease
  - $C0_2$ output ($VC0_2$): Modest numerical increase in both sexes (less than 5% change), but did not achieve statistical significance.
  - Ventilation rate: Modest numerical increase in both sexes (less than 5% change), but did not achieve statistical significance.
  - Respiratory exchange ratio (RER): No signficant change

- Blood analysis:
  - Partial pressure of oxygen ($P0_2$): No significant change
  - Partial pressure of carbon dioxide ($PC0_2$): No significant change
  - Hemoglobin saturation ($\%Sa0_2$): No significant change
  - pH of aterial blood: No significant change
  - All numerical changes in values were less than 5%. None of the changes achieved statistical significance.

- Metabolic assessment:
    - Lactate concentration: Numerical increase among both sexes, but changes were not statitiscally signicant. Increases were approximately 10%. Authors noted that 70% of men and 83% of women who were tested showed elevated [La] in the presence of H_2S compared to control conditions.

- Cardiovascular assessment:
    - Heart rate: No signficant change
    - Systolic blood pressure: No signficant change
    - Diastolic blood pressure: No signficant change
    - Rate pressure product: No signficant change

Subjective responses:

- Rating of perceived exertion: No signficant change
- No complaints of symptoms such as headache, undue fatigue, nausea or sore throat at the time of exposure and within 24 hours post-exposure.

*Statistically significant

Author's Conclusions:

- Exposure to 5ppm H_2S for 30min during submaximal exercise resulted in "*no significant differences…for the metabolic (oxygen uptake, carbon dioxide production, respiratory exchange ratio), cardiovascular (heart rate, blood pressure, rate pressure product), arterial blood (oxygen and carbon dioxide tensions, pH), and perceptual (rating of perceived exertion) responses in either sex*".
- "*..delta [La]…of the arterialized blood was higher in most of the subjects as a result of the H_2S exposure. However, the mean increase was not significantly different..nor was there a significant difference observed between the sexes*".
- "*5ppm* H_2S *did not significantly alter the HR (heart rate)…suggests that the sympathetic stimulus to the myocardium was unaffected..*" "*the..observations that systolic and diastolic blood pressures were unaltered…indicates that vascular control also was not significantly influenced.*"
- "*RPP (rate pressure product) is a valid predictor of $mV0_2$..Since RPP was unaffected..it is unlikely that $mV0_2$ was altered…This observation suggests that men and women with cardiovascular disease who may be exposed to H_2S …may not be at an increased risk*"
- "*In conclusion, the results of this study indicated that oral inhalation of 5 ppm H_2S for 30 minutes … does not significantly alter the metabolic, cardiovascular, and perceptual responses in healthy subjects. This concentration of the gas has no noticeable effect on arterial blood parameters asuch as oxygen and carbon dioxide tensions, or pH.*"
- "*Since there seem to be no adverse health effects as a result of the H_2S exposure,the evidence suggests that healthy men and women can safely work at moderate intensities in environments contaminated with 5 ppm H_2S.*"

Review – Assessment:

A. Subjects:	+ The number of subjects was adequate. + Subject profiles (gender, age, height, weight) were adequately described. + Clear exclusion criteria were utilized in the selection of volunteers. 'High risk' individuals, as defined by the Illinois Institute of Environmental Quality (1964), and those previously exposed were excluded. - The subjects were drawn from a very homogenous pool. They were primarily students in undergraduate or graduate programs at the testing institution. + Both genders were exposed. +/- The reported $V0_{2max}$ of subjects indicate that their aerobic fitness levels were slighlty higher than the average values reported for Canadians of their age and sex.

	+ Informed consent was given.
B. Exposure conditions:	+ The concentration of the gas to which subjects were exposed was continuously monitored and analytically confirmed. + The precise duration of exposure was recorded. +/- A mouthpiece was used to administer H_2S.
C. Equipment:	+ A full description of the exposure device and monitoring equipment was provided.
D. Procedural:	+ Subjects were exposed randomly. + Subjects were blinded to exposure concentrations. - It was unclear whether technicians monitoring the physiological parameters and conducting laboratory analyses were blinded. + Subjects served as their own controls, thus limiting confounding variables.
E. Data collection:	- Arterial blood was analyzed for lactate levels. + Although symptoms were both objective and subjective, assessment measures and scales were outlined. - Blood lactate levels are affected by the duration of time that has elapsed since the subject's last meal. Consequently, this period of time should have been standardized in the experimental procedure.
F. Data analysis:	- Confidence intervals were not provided. - Individual data were not provided to permit independent assessment of results. - Physiologic and metabolic parameters were reportedly monitored at 10-minute intervals during the 30-minute exposure period; however, only the findings at 30 minutes were provided.
G. Interpretations:	- Exposure was conducted during sub-maximal exercise. When extrapolating this study's findings to resting conditions this must be considered. - No comparisons between the physiological parameters of a normal person at rest, and the measured values, were conducted. - It is impossible to verify certain statements made by the authors due to the lack of raw data provided. (*e.g.*, whether in fact "*70% of the men and 83% of the women who were tested demonstrated an elevated delta [La]*" cannot be indendently assessed). - It is unclear what type of activities correspond with 50% of $V0_{2max}$.

Review - Summary:

Discussion of findings:

The focus of this study was on physiological and hematological responses of healthy men and women to acute H_2S exposure, to test the hypothesis *"that inhalation of 5ppm H_2S dring submaximal exercise would induce a significantly greater increase in [La] in women than in men"*. The hypothesis was not supported by the study findings. Although lactate concentrations tended to increase in both sexes, both inter-gender variability and intra-gender changes were insignificant. The findings indicate that oral inhalation exposure to 5 ppm H_2S for 30 minutes during submaximal exercise was without effect on metabolic, cardiovascular, arterial blood and perceptual responses. As noted earlier, (Bhambhani 1991 – Study ID 119), oxygen uptake and carbon dioxide output were unchanged by H_2S exposure during moderate exercise. Cardiovascular and vascular parameters measured were also unaltered. Overall no observable health effects or clinical symptoms were noted either during the course of the experiment or within 24 hr post-exposure.

Interpretation of the significance of the study findings should consider the following:

- Only healthy, young individuals were exposed. The aerobic fitness of the study subjects was reported to be higher than that of the average Canadian, as evidenced by relatively high VO_{2max} values. A higher level of aerobic fitness may provide protection against the toxic effects of H_2S (due to corresponding larger blood volumes and hemoglobin levels).
- Exposure was during submaximal exercise, and hence the ventilation rate of subjects was elevated.
- A mouthpiece was used to administer H_2S, hence, psychological implications of the odor of H_2S and the irritant capacity of the gas on the mucous

membranes of the nose and eyes were not assessed.

- No evidence was presented to indicate that women are more "at risk" than men as a result of H_2S exposure; however, the dose of H_2S inhlaed by the women in the study may have been less than that received by their male counterparts.
- The increase in blood lactate concentration noted for both sexes was relatively modest and did not achieve statistical significance. A shift in blood lactate level is a routine occurrence during normal diurnal rhythm and, increases occur normally in response to increased activity and physical exercise. Accordingly, the change in response to H_2S exposure is not unique.
- There was no indication that the feeding schedule of the test subjects was monitored and/or controlled as part of the study. The feeding schedule (*i.e.*, time elapsed between last meal and exposure) could affect the blood lactate levels.
- Lactate levels were analyzed in arterial blood. Arterial blood typically has a lower concentration than that of venous blood due to the removal of lactate by non-exercising tissue. Consequently, this is a less sensitive method of analysis.
- The absence of changes in heart rate, systolic blood pressure and distolic blood pressure *"suggests that men and women with cardiovascular diseasewho may be exposed to H_2S due to the nature of their occupations or otherwise, may not be at increased risk in such an environment".*

Confience index:

No significant omissions in the study design, conduct or reporting were noted, but the external validity of the study could have been improved by:

- Conducting trials under resting conditions as well as exercise.
- Participation of individuals more representative of the general population.
- Determination of lactate concentrations in venous blood.
- Monitoring of feeding schedule of subjects.
- Consideration in the data analysis of the total inspired volume of inhaled gas.
- Use of graded exposure concentrations to permit assessment of dose-responsiveness.

Review – Confidence index ranking:

High

Document Review

Author:	Bhambhani, Y; Burnham, R; Snydmiller, G; MacLean, I; Lovlin, R	**Internal ID:**	121
Title:	Effects of 10-ppm hydrogen sulfide inhalation on pulmonary function in healthy men and women.		
Year:	1996		
Abstract:	*"This study examined the acute effects of oral inhalation of 10 ppm hydrogen sulfide ($H_{2s}S$) inhalation (a concentration equal to its occupational exposure limit) on the pulmonary function in healthy men and women. Nine men and ten women consented to inhale medical air or 10 ppm H_2S for 15 minutes each during cycle exercise at 50% of their maximal aerobic power. Routine pulmonary function tests were administered at rest and immediately after the two exposure conditions. The results indicated no significant changes in any of the variables derived from the flow volume loop, maximum ventilation volume, and diffusion capacity of the lung for carbon monoxide in both genders. None of the subjects experienced any signs and symptoms as a result of H_2S exposure. It was concluded that oral inhalation of 10 ppm H_2S at an elevated metabolic and ventilation rate does not significantly alter pulmonary function in healthy men and women."*		
Objective:	To examine the acute effects of oral inhalation of 10ppm H_2S for15 min during submaximal exercise on the pulmonary function of healthy men and women.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0 or 10ppm (by mouthpiece)	Single/ 15 minutes	Both	Men: 24.7+/- 6.4yr Women: 21.8+/-3.0yr	19. (9 men; 10 women)	Healthy and physically active	10ppm	NA

Observations:

Objective responses:

- Within-gender observations (changes relative to control exposures):
  - Men:
    - -Forced vital capacity (FVC): 0.3% decrease
    - -Forced expiratory volume (FEV1): 0.2% decrease
    - -FEV1/FVC: 0.1% increase
    - -Peak expiratory flow rate (PEFR): 2% increase
    - -Forced expiratory flow at 25% to 75% of the FVC (FEF_{25-75}): 1.1% decrease
    - -Maximal ventilation volume (MVV): 0.5% increase
    - -Single-breath diffusion capacity of the lung for carbon dioxide (DLCO): 0.4% increase
    - - None of the changes achieved statistical significance
  - Women:
    - -FVC: 0.2% decrease
    - -FEV1: 1.7% decrease
    - -FEV1/FVC: 1.4% decrease
    - -PEFR: 2.8% decrease
    - -FEF: 3.1% decrease
    - -MVV: 1.4% increase

-DLCO: 0.3% decrease
-None of the changes achieved statistical significance

- Inter-gender observations:
  - FVC: 33% higher in men*
  - FEV_1: 31% higher in men*
  - FEV_1/FVC: 4% higher in women.
  - PEFR: 39% higher in men*
  - FEF_{25-75}: 13% higher in men*
  - MVV: 34% higher in men*
  - DLCO: 51% higher in men*

Subjective responses:

- None reported immediately following exposure or at 24 hours post-exposure.
- No indication of headache, nausea, throat irritation or other signs or symptoms that have been reported as a result of H_2S exposure.

*Statistically significant

Author's Conclusions:

- *"...acute oral inhalation of 10ppm H_2S for 15 minutes at a ventilation rate approximately four to five times the resting value does not adversely effect the pulmonary function profile in healthy men and women...subjects exposed to H_2S under such conditions do not experience any unusual symptoms."*
- *"None of the subjects experienced any unusual signs and symptoms immediately after the test, or during the following 24 hours".*
- *"It is possible that if the subjects could smell the gas, the pulmonary function results may have been different".*
- *"The results of this study demonstrate that oral inhalation of 10 ppm H_2S does not have any significant effects on the acute pulmonary function responses in healthy men and women".*

Review – Assessment:

A. Subjects:	+/- The number of test subjects was somewhat limited, especially since the findings were segregated according to sex. Nine males and ten females participated. + Subject profiles (*e.g.*, gender, age, weight, height) were adequately described. + Clear exclusion criteria were utilized for the selection of volunteers. 'High risk' individuals, as defined by the Illinois Institute of Environmental Quality (1964), and those previously chronically exposed were excluded. Individuals with respiratory, cardiovascular and/or metabolic disorders were excluded. +/- Participants were aerobically fit. Measures of their pulmonary function were notably better than those predicted for their age and gender. Hence, as stated by the author *"these individuals may not be representative of the population who may be exposed to H_2S"* + The study was conducted on both males and females. + Informed consent was given.
B. Exposure conditions:	+ The concentration of H_2S was continuously monitored and analytically confirmed. + The precise duration of exposure was recorded. +/- A mouthpiece was used to administer H_2S. This exposure method reduces detection bias but does not allow for detection of conjunctivitis, ocular injury and potential physiological responses triggered by smell.

	+	With the exception of temperature, all exposure conditions (airflow, humidity and concentration) were monitored.
C. Equipment:	+	A description of the exposure device and monitoring equipment was provided to permit reproducibility. Full details were available in a companion paper (Bhambhani and Singh, 1991 – Study ID 119)
D. Procedural:	+	Subjects were exposed randomly.
	-	The method of randomization (*e.g.*, table of random numbers, computer generated, etc.) was not noted.
	+	Exposure to the two exposure conditions (*i.e.*, 0 ppm vs. 10 ppm) was counterbalanced.
	+	Subjects were blinded to exposure conditions.
	+	Subjects served as their own controls, thus limiting confounding variables.
	+	A follow-up interview was conducted within 24 hours post-exposure.
	+	Measurements of pulmonary function were performed in duplicate to ensure reliability and reproducibility of findings.
E. Data collection:	+	Pulmonary function tests were completed within 3 minutes post-exposure.
	+	Measures of pulmonary function were assessed in duplicate to assess the precision of the assessment.
	-	Collection of subjective measures did not utilize a pre-determined scale.
F. Data analysis:	-	Confidence intervals were not provided.
	-	Individual data were not provided limiting the independent assessment of results.
G. Interpretations:	-	Exposure was conducted during sub-maximal exercise. The exercising ventilation volumes of subjects were 4 to 5 times that of resting subjects. When extrapolating the findings of this study to resting exposure, this should be considered.
	-	The ventilation rate was 41% higher in men than in women. Yet, no correction was made for the lower ventilation rate, and hence, exposure of the female subjects.
	-	The study subjects were aerobically fit and had pulmonary function profiles higher than those predicted for their age. Accordingly, these individuals may not be representative of the general population.

Review - Summary:

Discussion of findings:

Oral inhalation of 10ppm H_2S for 15 minutes at a ventilation rate four-to-five times the resting value, did not have any significant effect on pulmonary function, or general well-being of exposed healthy men and women. No significant changes in forced expiratory volume (FEV), forced vital capacity (FVC), peak expiratory flow (PEFR), maximum ventilation volume (MVV) or forced expiratory flow were recorded among the subjects following exposure to H_2S relative to control exposures. None of the subjects experienced any unusual clinical signs or symptoms, such as headache, nausea or sore throat, immediately following exposure or within 24 hours post-exposure.

Interpretation of the significance of the study findings should consider the following:

- Exposure was performed during submaximal exercise, and hence the ventilation rate of subjects was elevated. The inhaled dose of H_2S would be greater than that experienced by a resting individual.
- A mouthpiece was used to administer H_2S. Hence, psychological implications of the odor of H_2S and the irritant capacity of the gas on the mucous membranes of the nose and eyes was not assessed.
- Only young, aerobically fit adults were used as test subjects. As stated by the author *"these individuals may not be representative of the population who may be exposed to H_2S"*
- Exposure was of relatively short duration (*i.e.*, 15 minutes).

Confidence index:

The level of confidence assigned to the study findings and conclusions was high. No significant omissions in the study design, conduct or reporting were noted. However, interpretation of the significance and relevance of the findings could have been improved by:

- Conducting trials under resting conditions as well as during exercise to better represent activity patterns of the general population..
- Participation of individuals more representative of the general population. The test subjects were "aerobically fit".
- Use of more test subjects. Only 9 males and 10 females were tested.
- Consideration in the data analysis of the total inspired volume of inhaled gas.
- Use of graded exposure concentrations to permit assessment of dose-responsiveness.
- Extending the exposure duration beyond 15 minutes.
- Presentation of individual subject data to permit more meaningful interpretation of the findings.

Review – Confidence index ranking:

High

Document Review

Author:	Bhambhani, Y; Burnham, R; Syndmiller, G; Maclean, I	**Internal ID:**	122
Title:	Effects of 10ppm hydrogen sulfide inhalation in exercising men and women.		
Year:	1997		
Abstract:	*"This study examined the acute effects of 10 ppm hydrogen sulfide (H_2S) inhalation, a concentration equal to its occupational exposure limit, on the cardiovascular, metabolic, and biochemical responses in healthy volunteers. Fifteen men and 13 women completed two 30-minute exercise sessions at 50% of their maximal oxygen uptake, during which they inhaled medical air or 10 ppm H_2S in a blind manner. Arterial and finger-prick blood samples were obtained before and during the final minute of exercise. Muscle biopsies were withdrawn from the right vastus lateralis immediately after exercise. Cardiorespiratory measurements were monitored using an automated metabolic cart interfaced with an electrocardiogram and blood pressure apparatus. A significant decrease in oxygen uptake (VO_2), with a concomitant increase in blood lactate, was observed in men and women as a result of H_2S exposure. No significant changes were observed in arterial blood parameters and the cardiovascular responses under these conditions. Muscle lactate, as well as the activities of lactate dehydrogenase, citrate synthase, and cytochrome oxidase, were not significantly altered by H_2S exposure. However, there was a tendency for muscle lactate to increase and citrate synthase activity to decrease in both genders in the presence of H_2S. It appeared that 10-ppm H_2S inhalation reduced VO_2 during exercise, most likely by inhibiting the aerobic capacity of the exercising muscle. These findings question the scientific validity of the current occupational exposure limit for H_2S."*		
Objective:	To examine the effects of oral exposure to 10-ppm of H_2S on cardiovascular, metabolic, and biochemical parameters of healthy men and women during submaximal exercise.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age (years)	Number of subjects	Pre-trial health	NOAEL	LOAEL
0 or 10ppm (by mouthpiece)	Single/ 30 minutes. Exposures were performed in replicate once every three weeks.	Both	Men: 23.4+/-5.2 Women: 21.8+/-3.0	28. (15 men; 13 women)	Physically active	NA	10 ppm

Observations:

Objective responses:

- Pulmonary parameters:
    - O_2 uptake ($V0_2$): Reduced in both men and women. Change was less than 10% in both sexes. Response was reportedly inconsistent among subjects of both sexes, with 11 of 15 males and 9 of 13 females showing reduced VO_2.
    - $C0_2$ output (VCO_2): No significant change
    - Ventilation volume: No significant change.
    - Respiratory exchange ratio (calculated): Increased in both sexes.* Change was 6-12% and due to reduced oxygen uptake.

- Cardiovascular parameters:
    - Heart rate: No significant change
    - Systolic blood pressure: No significant change
    - Diastolic blood pressure: No significant change
    - Rate pressure product: No significant change

- Blood Analysis:

- Partial pressure of oxygen (PO_2): Modest numerical increase (less than 5%) in both sexes, but did not achieve statistical significance.
- Partial pressure of carbon dioxide ($PC0_2$): Modest numerical decrease in both sexes, but did not achieve statistical significance.
- $Sa0_2$%: No significant change
- pH: No significant change
- Arterial blood lactate concentration: Increased*. Change ranged from 70% (females) to 150% (males).

- Tissue analysis:
  - Muscle lactate concentration:
    - Men: 33% increase
    - Women: 16% increase
  - Lactate dehydrogenase: 7% reduced activity
  - Citrate synthetase: 7% reduced activity
  - Cytochrome oxidase:
    - Men: 16% reduced activity.
    - Women: 11% elevated activity
  - None of the changes in muscle tissue variables achieved statistical significance.

Subjective responses:

- Rating of perceived exertion: No significant change

*Statistically significant

Author's Conclusions:

- *"Oral inhalation of 10ppm H_2S significantly reduced the $V0_2$ and elevated blood [La] during 30 minutes of cycle exercise performed at 50% of $V0_{2max}$. The reduction in $V0_2$ was not a result of a decrease in oxygen availability, because oxygen-carrying capacity of the blood and oxygen-carrying capacity of the blood and oxygen transport were unaffected by H_2S exposure. The most likely reason for the decrease in $V0_2$ by 10ppm H_2S was a tendency for aerobic metabolism to be inhibited in the exercising muscle, as evidenced by decrease in the activities of CS in both genders and Cyt Ox in men."*
- *"It appeared that 10 ppm H_2S inhalation reduced VO_2 during exercise, most likely by inhibiting the aerobic capacity of the exercising muscle".*
- *"Cytochrome oxidase activity seemed to respond differently to H_2S exposure in men and women". "In men, Cyt Ox activity decreased by 16%, whereas in women it increased by 11%."*

Review – Assessment:

A. Subjects:	+/- The number of subjects was somewhat limited, especially since findings were segregated by sex. In addition, certain biochemical analyses were only conducted on a subset of samples (presumably due to limited tissue sample size). + Subject profiles (*e.g.*, gender, age, height, weight and hemoglobin levels) were adequately described. + Clear exclusion criteria were utilized in the selection of volunteers. 'High risk' individuals, as defined by the Illinois Institute of Environmental Quality (1964), and those previous exposed chronically were excluded. - The "*aerobic fitness levels* (of the subjects) *were above average expected for their age and sex*", hence subjects were not representative of the general population. + Informed consent was given.
B. Exposure conditions:	+ The exposure concentration was continuously monitored and analytically confirmed. + The precise duration of exposure was recorded.

	+/- A mouthpiece was used to administer H_2S. This exposure method reduces detection bias but omits potential physiological responses triggered by smell. + All exposure conditions (*e.g.*, airflow, humidity and concentration) except temperature, were monitored.
C. Equipment:	+ A full description of the exposure device and monitoring equipment was provided.
D. Procedural:	+ Subjects were exposed randomly. + Subjects and technicians conducting the biochemical analyses were blinded to the exposure conditions. + Exposures of each subject to the two exposure conditions (*i.e.*, 0 ppm vs. 10 ppm) were counterbalanced. + Subjects served as their own controls, thus limiting confounding variables + The single subjective measure (*i.e.*, rating of perceived exertion), utilized a pre-determined scale. - No follow-up exams were conducted.
E. Data collection:	+/- Analysis of LDH was conducted on only 14 biopsies in men and 12 biopsies in women. Lactate levels were determined in only fourteen biopsies in men and thirteen biopsies in women. No explanation for exclusion of certain subjects was provided. - Dilutions required to perform enzymatic assays might have lead to an underestimation of H_2S's effect upon the measured enzymes. + Duplicate measurements of lactate dehydrogenase, citrate synthetase and cytochrome oxidase levels were conducted. - Arterial blood was used to measure blood lactate. The use of venous blood would have been more sensitive. + Symptoms were primarily objective.
F. Data analysis:	- Confidence intervals were not provided. - Individual data were not provided to permit an independent assessment of results.
G. Interpretations:	- Exposure was conducted during sub-maximal exercise. This should be considered when extrapolating findings of this study to resting exposure. - No correction was made for the higher ventilation rate and hence exposure of women. - Subjects were young, aerobically fit and physically active, with higher than average VO_{2max} values (signifying above average aerobic fitness levels). This should be considered when extrapolating the findings from this study to the general population.

Review - Summary:

Discussion of findings:

Oral exposure of young physically active men and women to 10ppm H_2S for 30 minutes during sub-maximal exercise resulted in changes in selected pulmonary and metabolic parameters consistent with inhibition of aerobic metabolism. Specifically, oxygen uptake was significantly reduced and blood lactate concentration was significantly increased in both sexes. Modest changes in other biochemical parameters suggestive of a shift from aerobic to anaerobic metabolism also were reported, including an increase in muscle lactate concentration and a decrease in citrate synthetase activity in both sexes, and inhibition of cytochrome oxidase in the male subjects. However, the latter changes did not achieve statistical significance. Changes in certain biochemical parameters argued against any shift toward anaerobic metabolism, including reduced muscle lactate dehydrogenase activity in both sexes, and increased cytochrome oxidase activity among the female subjects.

Cardiovascular parameters were unaffected by exposure, with no changes observed in heart rate, systolic blood pressure, diastolic blood pressure, and rate pressure product. Similarly, arterial blood parameters remained unchanged following treatment, with the partial pressures of oxygen and carbon dioxide, blood pH, and hemoglobin saturation showing no differences between control and H_2S exposures.

Interpretation of the significance of the study findings and their relevance to the general population should consider the following:

- Subjects were exercising to 50% of their aerobic capacity during exposure. Accordingly, the inhaled dose of H_2S was higher than that which would be expected to occur under normal daily activity patterns in which resting conditions are common.
- A mouthpiece was used to administer H_2S. Hence, possible pychological and/or physiological responses to the odor of H_2S and the irritant capacity of the gas on mucous membranes of the nose and eyes were not assessed.
- The observed changes in biochemical parameters were *suggestive* of a shift from aerobic to anaerobic metabolism following exposure to 10 ppm H_2S; however, certain of the responses were not statistically significant, other responses were not consistent between the sexes, and still other responses signalled no shift from aerobic metabolism. Given the inconsistencies and the modest nature of certain of the changes, it is difficult to assess the significance of the findings, even on the basis of weight-of-evidence.
- The changes in biochemical parameters were not accompanied by any shifts in arterial blood parameters or changes in cardiovascular function. Nor were any clinical symptoms consistent with H_2S expsoure recorded. Thus, it would appear that the changes produced by the exposure to 10 ppm H_2S were confined to the cellular level. Moreover, the cellular changes were modest and did not reach the level required for clinical expression of toxicity.
- The reportedly observed shift from aerobic to anaerobic metabolism is not unique to H_2S exposure. Rather, the same shift will occur with increased physical activity (*e.g.,* exercise) and as part of normal diurnal rhythms (*e.g.,* overnight fasting).
- The aerobic fitness levels of the subjects were above average for their age and sex. Hence, the subjects were not likely representative of the general population, and perhaps at lower risk from H_2S exposure due to higher oxygen capacity and elevated blood volumes.
- Exposure was of short duration (*i.e.*, 30 minutes).

Confidence index:

A high level of confidence was assigned to the study findings and conclusions. No significant weaknesses in study design, conduct and/or reporting were evident. However, interpreation of the significance of the findings and their relevance to the genral population might have been improved by:

- Conducting the trials under resting conditions as well as during physical exercise.
- Participation of individuals more representative of the general population.
- Consideration in the data analysis of the total inspired volume of inhaled gas.
- Use of graded exposure concentrations to permit assessment of dose-responsiveness.
- Determinination of the maximal power output to assess fatigue [as completed previously in Bhambhani and Singh (Bhambhani and Singh 1991 – Study ID 119)].
- Extending the exposure duration beyond 30 minutes.
- Presentation of individual subject data to provide for more meaningful interpretation of the study findings.

Review – Confidence index ranking:

High

Document Review

Author:	Dorman, DC; Brenneman, KA: Struve, MF; Miller, KL; James, RA; Marshall, MW* *Note: Identical results reported in: Chemical Industry Institute of Toxicology* Dorman, DC; Brenneman, KA; Struve, MF; Miller, K; James, RA; Marshall, MW (Study ID - 594) Final report: A vapor inhalation reproductive toxicity, developmental toxicity, and developmental neurotoxicity study in Crl:CD®(SD)CR rats exposed to hydrogen sulfide. 1999	**Internal ID:**	149
Title:	Fertility and developmental neurotoxicity effects of inhaled hydrogen sulfide in Sprague-Dawley rats		
Year:	2000		
Abstract:	*"In this study, we examined whether perinatal exposure by inhalation to hydrogen sulfide (H_2S) had an adverse impact on pregnancy outcomes, offspring prenatal and postnatal development, or offspring behavior. Virgin male and female Sprague-Dawley rats (12 rats/sex/concentration) were exposed (0, 10, 30, or 80 ppm H_2S; 6 h/day, 7 days/week) for 2 weeks prior to breeding. Exposures continued during a 2-week mating period (evidence of copulation = gestation day 0 = GD 0) and then from GD 0 through GD 19. Exposure to dams and their pups (eight rats/litter after culling) resumed between postnatal day (PND) 5 and 18. Adult male rats were exposed for 70 consecutive days. Offspring were evaluated using motor activity (PND 13, 17, 21, and 60 ± 2), passive avoidance (PND 22 ± 1 and 62 ± 3), functional observation battery (PND 60 ± 2), acoustic startle response (PND 21 and 62 ± 3), and neuropathology (PND 23 ± 2 and 61 ± 2). There were no deaths and no adverse physical signs observed in F0 male or female rats during the study. A statistically significant decrease in feed consumption was observed in F0 male rats from the 80-ppm H_2S exposure group during the first week of exposure. There were no statistically significant effects on the reproductive performance of the F0 rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. Exposure to H_2S did not affect pup growth, development, or performance on any of the behavioral tests. The results of our study suggest that H_2S is neither a reproductive toxicant nor a behavioral developmental neurotoxicant in the rat at occupationally relevant exposure concentrations ($\leq$ 10 ppm)"*		
Objective:	To evaluate whether repeated 6-h daily exposure of male and female rats to H_2S atmospheres of 0, 10, 30, or 80ppm resulted in reproductive or developmental toxicity.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 10ppm; 30ppm; 80ppm (whole body)	Repeated/ 6 hours per day as follows: i) during two-week pre-breeding period (F_O males and females); ii) during two-week mating period (F_O males and females); iii) during gestation, from Gestation Day (GD) 0 to 19 (F_O females; and, iv) from post-natal day (PND) 5 through 18 (F_O females and F_1 pups). Note that daily exposure of F_O males continued until they were exposed for at least 70 days.	Rat	Sprague - Dawley	8 weeks upon arrival; 10 weeks at start of treatment .	Both	12 rats/sex/ exposure group to yield at least 8 pregnant dams per exposure concentration.	Healthy	NA	10ppm

Observations:

- F_0 generation:
  - Feed consumption:
    - -Male: Decrease during first week of exposure at 80ppm*
    - -Female: Decrease during first week of exposure at 30 ppm and 80ppm
    - -Decreased feed consumption did not persist beyond the initial pre-mating period.
  - Body weight:
    - -Male: 5-6% decrease at 80 ppm, which persisted throughout 10-week exposure period.
    - -Female: 5-6% decrease at 80 ppm.
  - Reproductive performance (based on consideration of mating index, fertility index, post-implantation losses, number of late resorptions, length of gestation, number of stillbirths, litter size, number of females with live pups, and number of implants per pregnant female):
    - -Male: No change
    - -Female: No change
  - Clinical observations: No treatment-related clinical signs of toxicity. No deaths occurred.
  - Organ weights:
    - - Males: No change in absolute or relative weights of brain, heart, kidneys, liver, lungs, spleen, epididymidies, prostate, testes, seminal vesicles, and coagulating glands.
      Decrease in absolute and relative weights of adrenal glands at 10 ppm* and 80 ppm*
    - - Females: No change in absolute or relative weights of adrenal glands, brain, kidneys, liver, spleen, and uterus.
      Decrease in relative weight of ovaries at 10 ppm*

  Pathology:
    - - Males: Mild to marked sensory neuron loss and basal cell hyperplasia in olfactory mucosa (exposure concentration not specified).
      Isolated cases of seminiferous tubular degeneration and associated changes in epididymis at 80 ppm.
    - - Females: No remarkable findings. Isolated cases of ovarian cysts and squamous metaplasia of the endometrium at 10 ppm and 30 ppm
- F_1 pups:
  - Structural malformations:
  - - Isolated instances of external malformations among pups borne to dams exposed to H_2S. Incidence was rare, and described as follows:
    - *"No statistically significant increase in structural malformations was observed in this study. Malformations, however, were only observed in newborn pups from dams that were exposed to H_2S."*
    - *"No dose-response relationship was observed for any external alteration, because malformations were observed in litters from all treatment groups."*
  - Pup body weight: No change.
  - Pup development: Unaffected (based on consideration of onset of pinnae detachment, incisor eruption, negative geotaxis, eyelid separation, vaginal patency, and/or preputial separation.
  - Pup survival: No change

- Behavioral effects:
  - Motor activity:
    - *"Statistically significant changes in motor activity were observed during the preweaning and postweaning period only in animals exposed to 10ppm H_2S...This effect was not treatment related because no dose-response relationship was observed for this end point, and it was only observed at one time point in one gender."*
  - Acoustic startle response: No change.

  - Passive avoidance testing: No change.
  - Functional observation battery (FOB) assessment: No change (based on examination for signs of ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration, or abnormal pupil reflex, as well as assessment of acoustic response, tail pinch response, approach response, visual placing response, hind and forelimb grip strength, hind-limb splay, urine and feces production, rearing behavior, neuromuscular dysfunction and body appearance).

- Organ weights: No change in weights of all organs examined (*i.e.*, adrenal glands, brain, heart, kidneys, liver, lungs, spleen, testes).

- Neuropathology: No histological abnormalities in brain, spinal cord or peripheral nerves.
  Increase in brain length in male pups at PND 23 at 10 ppm*

*Statistically significant

Author's Conclusions:

- *"The results of our study suggest that H_2S is neither a reproductive toxicant nor a behavioral developmental neurotoxicant in the rat at occupationally relevant exposure concentrations (<10ppm)."*

Review – Assessment:

A. Test animals:	+ Number of test animals allocated to each exposure group (*i.e.*, n=12 per sex) was adequate. + The principle characteristics of test animals (e.g., specie, strain, age, sex, pre-test health status, weight variation and source) were specified. + Pre-test health status was confirmed by a veterinarian during the acclimatization period. Representative animals were subjected to both a fecal examination and a serum antibody analysis. - Weight variation of males (26%) and females (40%) is above the 20% suggested by OECD guidelines.
B. Exposure conditions:	+ The source of the H_2S gas was provided. +/- An analysis to detect impurities in the hydrogen sulfide gas was conducted. Propylene (0.19%), propane (<0.01%) and water (0.01%) were detected. + Analyses were conducted after the study to demonstrate the stability of the gas. + The rational behind the choice of exposure concentrations was fully outlined. + The gas concentration in exposure chambers was equilibrated before test animals were placed inside. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. + Airflow was monitored continuously. + Gas concentrations within the chamber were monitored and actual concentrations were analytically determined. Deviations from target concentrations were uniformly less than 10%. + The potential for adsorption of H_2S onto the cage litter was investigated, and no detectable adsorption was found. + No H_2S was detected in the control chamber +/- Measurements of tube concentration, temperature, and humidity were recorded during each exposure period. However, temperature, and humidity were only determined in one exposure cylinder per exposure group. - Animals were transferred from housing cages to glass exposure cylinders and then returned to housing cages after exposure. This step could result in additional handling and stress.

C. Housing/Feeding:	+ The dimensions and the material from which cages were constructed were noted. + Commercial pelleted rat feed and water were fed *ad libitum* to test animals. The feeding schedule was tightly regulated and complied with OECD guidelines. + An analysis of the chemical composition and possible contamination of the rodent feed and drinking water was conducted by suppliers and the results were recorded in the study records. + Animals were housed in an environmentally controlled room (*e.g.*, 64-79F; 30-70% humidity; 12hr photoperiod) + The type of bedding (*i.e.*, cellulose fiber chips) was specified. + Animals were individually housed, except during the mating period.
D. Equipment:	+ All exposure, monitoring, and analysis equipment was thoroughly described. + Instruments were routinely calibrated. Temperature and humidity were monitored every 30 min using a thermistor and a humidity probe. + Limit of detection of instrumentation was noted.
E. Procedural:	+ Animals were acclimatized for two weeks prior to exposure. - It was not noted whether animals were acclimatized to the noise and confinement within the exposure chamber prior to exposure. + Rats were identified by both eartag and transponder. + Animals were randomly assigned to exposure groups, and the order by which samples were analyzed was also random. + Controls were also placed within the chamber to account for physiological responses associated with stress. + Pups and dams were not separated between postnatal day 5 through 18, avoiding any impact of stress due to maternal separation. + Clinical examinations were conducted and recorded daily, both prior and after each exposure period. This record included the severity, duration and time of onset of symptoms. + Observations for mortality were made twice daily. + Body weights were determined and recorded weekly until confirmation of mating. + Feed consumption was measured weekly (except during the period of cohabitation). + A trained neurotoxicology technician conducted the FOB examination. + A single technician performed all FOB examinations to maintain consistency. + Animals were euthanized by $C0_2$ asphyxiation. + This study was performed in compliance with the OECD Guideline for testing of chemicals 421, and in compliance with the Good Laboratory Practices (GLP) Standards of the Environmental Protection Agency's Toxic Substances Control Act.
F. Data collection:	+ Appropriate endpoints were examined, and the assessment was thorough. Records included the clinical nature, duration, severity, onset and dose-response relationship for each symptom. + The timing and frequency of assessments was strictly regulated. + Scales used to assess developmental landmarks were referenced (Adams *et al.* 1985) +/- All behavioral tests were solely conducted on one male and one female per litter. - Motor activity was measured in the same animals (one male and one female) - Passive avoidance was only assessed on one male and one female per litter
G. Data analysis:	+ Statistically analyses were performed using a commercial software program, SAS Statistical software. + The statistical analysis adjusted for the large body weight distribution of test animals. + *"The data for quantitative, continuous variables (e.g., parental and pup body weight, organ weights, feed consumption, etc.) were intercompared for the exposure and control groups by tests for homogeneity of variance (Levene's test), 2-way fixed effects (dose and sex) analysis of variance (ANOVA), and Dunnett's multiple comparison procedure for significant ANOVAs."*

	+ *"Incidence data were compared using the appropriate statistical test, generally Fisher's Exact Test."*
H. Interpretations:	+ The primary focus of this report was addressed. + The potential impact of confounding variables (*e.g.*, sex, age, weight variation, chamber stress, bias, etc) was limited by either the experimental design or statistical analysis. - The following statements were contradictory, *"H_2S neither affected sperm production nor morphology"* ; *"a large percentage of abnormal sperm was observed in two F_0 rats form the 30- and 80-ppm exposure groups. Abnormal sperm accounted for 29 and 73% of all sperm analyzed from the 30- and 80-ppm exposed rats"*

Review - Summary:

Discussion of findings:

No clear evidence of reproductive or developmental toxicity was presented following inhalation exposure of male and female rats to 0, 10,30, or 80 ppm of H_2S under controlled conditions for 6 hours per day during the course of a two-week pre-breeding period, a two-week mating period, the gestation period, and a three-week postnatal period. Reproductive performance of the F_0 parental animals was unaffected by treatment, as evidenced lack of exposure-related changes in mating index, fertility index, post-implantation losses, number of late resorptions, number of stillbirths, average length of gestation, litter size, and average number of implants per pregnant dam. Development of the F_1 pups also was unaffected by treatment, with no significant exposure-related changes in developmental landmarks (*i.e.*, pinnae detachment, incisor eruption, eyelid separation, negative geotaxis, vaginal patency and/or preputial separation) and behavioral parameters (*i.e.*, motor activity, passive avoidance, functional observation battery and accoustic startle). Postmortem examination of the F_0 and F_1 animals revealed no remarkable findings attributable to treatment upon necropsy and microscopic examination of selected tissues. Apart from a modest reduction in body weight and initial decreased feed consumption among the high-dose F_0 males, any differences in reproductive, developmental or pathological parameters between the treated rats and the corresponding controls were generally isolated, minor in nature, non-dose-related and/or of unknown biological significance. Accordingly, the differences were deemed to be non-treatment-related.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The study was performed, to the extent possible, in accordance with the recommendations contained in OECD Guideline 421 for reproductive and developmental toxicity, *and* was conducted in compliance with Good Laboratory Practice (GLP) requirements. This heightens the level of confidence that can be assigned to the study findings and conclusions.
- The modest reduction in feed consumption noted among the F_0 parental animals at the initiation of treatment is not uncommon and represents a normal response to a chemical stressor in laboratory rodents. The response was transient (*i.e.*, it did not persist beyond the initial pre-breeding period) and was confined to the high-dose males and mid- and high-dose females only. The response achieved statistical significance only among the high-dose males. It is perhaps noteworthy that the initial decline in feed consumption likely contributed to the small decrease in body weight reported for the F_0 males and females exposed to 80 ppm H_2S.
- It was reported that no statistically significant increase in structural malformations was observed among the F_1 pups, but that malformations were only observed in newborn pups from dams that were exposed to H_2S. Careful review of the nature and incidence of the malformations revealed no evidence that the structural defects were related to treatment. In all cases, the malformations were isolated events, occurring randomly among all treated groups, with no indication of dose-responsiveness. The majority of affected pups were from a single litter, and the most common structural defect was described as *"skin and dermis detachment"*, which was determined to have a genetic origin.
- It was reported that H_2S neither affected sperm production nor morphology. However, abnormal sperm were determined upon andrological examination in a single F_0 male from each of the mid- and high-dose exposure levels. In addition, routine histopathology revealed instances of seminiferous tubular degeneration and associated changes in the epididymis among the F_0 males exposed to 80 ppm of H_2S. The toxicological significance of these findings is unknown. The responses were isolated and overall sperm production was unaffected by treatment.
- Other differences in reproductive, developmental or pathological parameters observed between the treated rats and the corresponding controls were

generally isolated, minor in nature, and non-dose-related. Accordingly, they should not be regarded as being treatment-related.

Confidence index:

The study was conducted in compliance with OECD Guideline 421 for reproductive and developmental toxicity, and in compliance with Good laboratory Practice (GLP) requirements. The experimental design and conduct were well described. Reporting of findings was judged to be complete.

Review – Confidence index ranking:

High

Document Review

Author:	Dorman, DC; Moulin, FJM; McManus, BE; Mahle, KC; James, RA; Struve, MF	**Internal ID:**	151
Title:	Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium		
Year:	2002		
Abstract:	*"Hydrogen sulfide (H_2S) is an important brain, lung, and nose toxicant. Inhibition of cytochrome oxidase is the primary biochemical effect associated with lethal H_2S exposure. The objective of this study was to evaluate the relationship between the concentration of sulfide and cytochrome oxidase activity in target tissues following acute exposure to sublethal concentrations of inhaled H_2S. Hindbrain, lung, liver, and nasal (olfactory and respiratory epithelial) cytochrome oxidase activity and sulfide concentrations were determined in adult male CD rats immediately after a 3-h exposure to H_2S (10, 30, 80, 200, and 400 ppm). We also determined lung sulfide and sulfide metabolite concentrations at 0, 1.5, 3, 3.25, 3.5, 4, 5, and 7 h after the start of a 3-h H_2S exposure to 400 ppm. Lung sulfide concentrations increased during H_2S exposure and rapidly returned to endogenous levels within 15 min after the cessation of the 400-ppm exposure. Lung sulfide metabolite concentrations were transiently increased immediately after the end of the 3-h H_2S exposure. Decreased cytochrome oxidase activity was observed in the olfactory epithelium following exposure to $\geq$ 30 ppm H_2S. Increased olfactory epithelial sulfide concentrations were observed following exposure to 400 ppm H_2S. Hindbrain and nasal respiratory epithelial sulfide concentrations were unaffected by acute H_2S exposure. Nasal respiratory epithelial cytochrome oxidase activity was reduced following acute exposure to $\geq$ 30 ppm H_2S. Liver sulfide concentrations were increased following exposure to $\geq$ 200 ppm H_2S and cytochrome oxidase activity was increased following inhalation exposure to $\geq$ 10 ppm H_2S. Our results suggest that cytochrome oxidase inhibition is a sensitive biomarker of H_2S exposure in target tissues, and sulfide concentrations are unlikely to increase postexposure in the brain, lung, or nose following a single 3-h exposure to $\leq$ 30 ppm H_2S."*		
Objective:	To evaluate the relationship between the concentration of sulfide and cytochrome oxidase activity in target tissues following acute exposure to sublethal concentrations of inhaled H_2S in rats. Work was also directed at determining whether increased sulfide deposition or enhanced inhibition of cytochrome oxidase could account for the increased sensitivity of the rodent olfactory epithelium to H_2S inhalation. Note that a series of experiments were performed involving acute (*i.e.*, single), subacute (*i.e.*, 5 –day), and subchronic (*i.e.*, 70-day) inhalation exposures to H_2S. The present review was directed primarily at the assessment of the findings from the acute and subacute studies.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; **10**ppm; **30**ppm; **80**ppm; 200ppm; 400ppm (nose only)	(a) Single/ 3 hours (b) Repeated/ 3 hours a day for five days [(c) Repeated/ 6 hours a day for 70 days]	Rat	CD	9 to 10 weeks old	Male	266. 6 per exposure concentration for each experiment.	Not specified.	10ppm (?)	30ppm

Observations:

- Tissue sulfide concentrations (after single 3-hour exposure):
  - Hindbrain:
    - 200ppm: 7.4% decrease
    - 400ppm: 5.8% decrease
  - Respiratory epithelium:
    - 200ppm: 20.8 % decrease
    - 400ppm: 58% increase

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  - Olfactory epithelium:
    - 200ppm: 12% decrease
    - 400ppm: 46% increase*
  - Lung:
    - **10**ppm: 20% decrease
    - **30**ppm: 19% increase
    - **80**ppm: 44% increase*
    - 200ppm: 80% increase*
    - 400ppm: 63% increase*
  - Liver:
    - **10**ppm: 101% increase
    - **30**ppm: 35% increase
    - **80**ppm: 56% increase
    - 200ppm: 181% increase*
    - 400ppm: 1815 increase*

- Sulfide metabolite concentrations (*i.e.*, sulfite, sulfate, and thiosulfate) in lungs:
  - Significant increase was observed in rats exposed to 400ppm H_2S and occurred 15 min after the end of the 3-hour exposure. Levels declined to pre-exposure concentrations within minutes.

- Cytochrome oxidase activity (after single 3-hour exposure):
  - Hindbrain:
    - 200ppm: 19% increase
    - 400ppm: 7% increase
  - Respiratory epithelium:
    - **30**ppm: 51% decrease*
    - **80**ppm: 59% decrease*
    - 200ppm: 25% decrease*
    - 400ppm: 24% decrease*
  - Olfactory epithelium:
    - **30**ppm: 16% decrease*
    - **80**ppm: 18% decrease*
    - 200ppm: 23% decrease*
    - 400ppm: 23% decrease*
  - Lung:
    - **10**ppm: 6% decrease
    - **30**ppm: 11% decrease*
    - **80**ppm: 17% decrease*
    - 200ppm: 19% decrease*
    - 400ppm: 34% decrease*
  - Liver:
    - **10**ppm: 33% increase*

- **30**ppm: 48% increase*
- **80**ppm: 35% increase*
- 200ppm: 38% increase*
- 400ppm: 39% increase*

- Cytochrome oxidase activity (after 5-day exposure)
  - Respiratory epithelium:
    - **30**ppm: 19% decrease
    - **80**ppm: 41% decrease
    - 200ppm: 27% decrease
    - 400ppm: 16% decrease
  - Olfactory epithelium:
    - **30**ppm: 34% decrease*
    - **80**ppm: 25% decrease*
    - 200ppm: 38% decrease*
    - 400ppm: 45% decrease*

*Statistically significant

Author's Conclusions:

- *"...end-of exposure lung sulfide concentrations were highly correlated with the amount of H_2S in the atmosphere..."*
- *"...elevated lung sulfide concentrations were observed following a single 3-h H_2S exposure to $\geq$ 80ppm...In contrast, decreased cytochrome oxidase activity was observed in the lung after a 3-h exposure to $\geq$ 30ppm H_2S...This finding indicates that cytochrome oxidase inhibition is a more sensitive biomarker of H_2S exposure than tissue sulfide concentrations."*
- *"Lung sulfide concentrations rapidly returned to preexposure levels within minutes after the end of a 3-h exposure to 400ppm H_2S, suggesting that rapid pulmonary elimination or metabolism of sulfide occurs*
- *...increase in sulfide metabolite concentrations occurred coincidentally with the rapid decrease in lung sulfide concentration...suggests that the concentration of sulfide to sulfate may become less effective as the concentration of sulfide increases "*
- *...increased sulfide concentrations and cytochrome oxidase inhibition in the upper respiratory tract from H_2S-exposed rats. Although our data showed that olfactory, but not respiratory, epithelial sulfide concentrations were significantly elevated following exposure to 400ppm H_2S, the observed differences in end-of-exposure tissue sulfide concentrations are unlikely to be toxicologically significant"*
- *"Our data suggest that the regenerated respiratory epithelium becomes resistant to H_2S-induced cytochrome oxidase inhibition"*
- *"...dose-response data for cytochrome oxidase activity in the nasal tissue demonstrates some inconsistencies...may indicate that some compensatory changes are occurring in the epithelium in response to H_2S exposure."*
- *"rats exposed...had significantly elevated hepatic cytochrome oxidase activity...The biological significance of this observation is unclear, but it implies that respiration in the liver is not inhibited by H_2S at these treatment concentrations, and possibly, that H_2S detoxification processes in this organ require additional energy demands, as reflected by increased respiratory cytochrome activity."*
- *"Our results suggest that acute exposure to low concentrations ($\geq$ 30ppm) of H_2S is associated with cytochrome oxidase inhibition in the lung and nose. Inhibition of cytochrome oxidase often occurred in the absence of elevated tissue sulfide concentration. These data suggest that cytochrome oxidase inhibition is a more sensitive biomarker of H_2S exposure than is tissue sulfide concentration."*

Review – Assessment:

A. Test animals:	+/- Six rats per exposure concentration were employed in each portion of the experiment. The number of animals was judged to be adequate for the purposes of the investigation, but did not satisfy guideline recommendations for acute and subacute toxicity studies (*i.e.*, 10 to 20 rats per exposure level). +/- The principle characteristics of test animals (*e.g.*, specie, strain, age, sex and source) were adequately reported; however, information pertaining to weight variation and pre-test health status was not provided. - Only male rats were exposed.
B. Exposure conditions:	+ Source of H_2S gas was indicated +/- Nose-only exposure was used. + Target exposure concentrations were analytically confirmed at least six times during each 3-h exposure.
C. Housing/Feeding:	+ The material from which cages were constructed was noted (polycarbonate). However, the dimensions of these cages were not included in the description. + Test animals were individually-housed. Therefore, clear observation of each animal was possible. + Commercial pelleted rat feed and deionized filtered tap water were provided ad libitum, except during exposure. + Animals were housed in an environmentally controlled room (*e.g.* 18.5 to 21.5°C; 40-70% humidity; 12 h light-dark cycle) - Airflow was not noted. + Bedding type was specified. + Study was conducted according to federal guidelines for the care and use of laboratory animals (NRC, 1996) and under the supervision of the CIIT Institutional Animal Care and Use Committee.
D. Equipment:	+ Most exposure, monitoring and analysis equipment was adequately described in Struve (2001- Study ID 296),
E. Procedural:	- If test animals were acclimatized prior to exposure this was not recorded. - Unclear if rats were randomly assigned to exposure groups. + Control exposures were performed. - It is unclear whether technicians and handlers were blinded to exposure conditions. +/- Method of sacrifice varied (*i.e.*, $C0_2$ asphyxiation vs. decapitation) + All biochemical assay methods were referenced. + Sulfide and sulfide metabolite concentrations were analytically assessed simultaneously in the same samples due to the use of both an amperometric analytical cell and a conductivity detector. - Cytochrome oxidase activity and sulfide concentrations should have been assessed in the same sample. It was unclear whether this was done. + The authors checked the validity of their analytical method by comparing the experimental tissue sulfide values experimentally determined to those found in the literature.
F. Data collection:	- Individual data were not provided for each test animal, thereby limiting independent assessment of the findings. - Conventional measures of toxicity were not performed (*i.e.*, clinical observations, body weights, feed consumption, clinical pathology, organ weights, gross pathology, and/or histopathology)
G. Data analysis:	+ Statistical analysis was conducted using a standard statistical package + *"Time-course and dose-response data were evaluated by one-way analysis of variance (ANOVA) followed by a comparison with the preexposure (control) group using Dunnett's test."* + *"The distribution of all data was tested for normality using the Shapiro-Wilk test before analysis."* + *"Linear regression correlations were performed according to standard statistical procedures and tested by analysis of variance."*
H. Interpretations:	+ The objective of the study was addressed by the experimental procedure.

	+ The authors state that *"Few studies have examined sulfide concentrations in lung, brain, or other target tissues following H_2S inhalation"*. This comment addresses the novelty of an investigation defining the relationship between H_2S exposure concentrations, tissue sulfide concentrations and cytochrome oxidase activity in the hindbrain, lung, and nose .

Review - Summary:

Discussion of findings:

Nose-only exposure of male rats to H_2S on either an "acute" or "subacute" basis under controlled conditions resulted in biochemical changes in selected target tissues, as evidenced by shifts in tissue sulfide content, cytochrome oxidase activity and/or the concentrations of sulfide metabolites. Changes were most pronounced in the lung and nasal olfactory epithelium, with more modest effects observed in the liver and nasal respiratory epithelium. The hindbrain was unaffected. The inhibition of cytochrome oxidase was determined to be a decidedly more sensitive marker of H_2S toxicity compared to tissue sulfide levels. In most instances, the nature, time-course and dose-responsiveness of the changes were consistent with the mechanism of action of H_2S, with the effects on cytochrome oxidase activity and tissue sulfide levels being inversely related. Inhibition of cytochrome oxidase was evident following exposure to H_2S at concentrations as low as 30 ppm, with the response being similar in the lung, nasal olfactory epithelium and nasal respiratory epithelium. In the liver, an opposite response was observed, with the activity of cytochrome oxidase *increased* in response to exposure to concentrations as low as 10 ppm. Tissue sulphide content was affected only at concentrations of 80 ppm and greater.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The study was directed primarily at understanding the dosimetry and pharmacodynamics of H_2S at the cellular level, and not at examining the health effects *per se* following H_2S exposure.
- The mode of administration of H_2S involved nose-only exposure, with the rats restrained. This mode of exposure is largely "foreign" to the human condition, and perhaps weakens the clinical relevance of the findings.
- The only change noted following exposure to 10 ppm of H_2S was an *increase* in cytochrome oxidase activity in the liver. Although the change was dose-responsive, its biological significance is unknown.
- In the absence of conventional measures of toxicity (*i.e.*, clinical observations, clinical pathology, gross pathology, histopathology), the clinical significance of the observed changes in tissue sulphide content and cytochrome oxidase activity is nebulous. The changes are consistent with the mechanism of action of H_2S, but in the absence of clinical or pathological correlates, it is difficult to assign clinical significance to the findings.
- The findings suggest that post-exposure recovery of the lung and nasal respiratory epithelium is relatively rapid, with tissue sulphide content returning to pre-exposure levels in the lung within minutes following exposure. Regeneration of the nasal respiratory epithelium also was reported to occur quickly. However, recovery of the nasal olfactory epithelium was more protracted, possibly reflecting an increased sensitivity of the neuroepithelium to H_2S exposure.
- The study used male rats only, thereby introducing at least two confounding variables in terms of extrapolating the findings to the human condition. First, the lack of use of the female sex limits the application of the findings. Second, the morphological architecture of the rat nasal passages is complex and the observed changes in the nasal epithelium may differ from those that might be experienced in humans.

Confidence Index:

The level of confidence assigned to the study findings and conclusions was moderate. In general, the study was adequately designed, conducted and reported. Interpretation of the findings by the authors was thorough and reasonable. The clinical relevance of the findings might have been strengthened by:

- Use of both sexes of test animals
- Use of whole-body exposure.
- Addition of conventional measures of toxicity to permit better assessment of the clinical significance of the observed biochemical responses.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Elovaara, E; Tossavainen, A; Savolainen, H	**Internal ID:**	155
Title:	Effects of subclinical hydrogen sulfide intoxication of mouse brain protein metabolism.		
Year:	1978		
Abstract:	*"Exposure of adult female mice to 100 ppm hydrogen sulfide for 2 h resulted in a marked decrease in incorporation of labeled leucine in cerebral protein and myelin 24 and 48 h after the exposure. The reduced incorporation was accompanied by decreased lysosomal acid proteinase activity at the same time. The incorporation increased to the control level 72 h after the exposure whereas the lysosomal enzyme activity remained below the control range at the end of the follow-up period. The changes were distinct from carbon monoxide-induced changes and might have resulted from the inhibition of cerebral cytochrome oxidase activity. The delay of maximal effects to the third post exposure day might pose a potential toxicological hazard in repeated exposure to higher hydrogen sulfide concentrations."*		
Objective:	To investigate the effect of "acute" exposure to 100ppm H_2S on protein metabolism in the mouse brain.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
0 ppm;100ppm (whole body)	Single/ 2 hours	Mice	CB-20	Adult	Female	40. (30 exposed; 10 control)	Not specified.	NA	100ppm

Observations:

- Clinical observations: Agitation during first 20 min, but mice subsequently calmed and no observable difference in activity from controls was noted during latter stages of exposure or during the follow-up period. No deaths occurred.

- Homogenate of left cerebral hemisphere:
  - Protein content: No significant change
  - RNA levels: No significant change
  - Leucine uptake:
    - 2 hours post-exposure: 12% increase
    - 6 hours post-exposure: 4% increase
    - 24 hours post-exposure: 20% decrease*
    - 48 hours post-exposure: 26% decrease*
    - 72 hours post-exposure: 7% decrease
  - Acid proteinase activity:
    - 2 hours post-exposure: 6% decrease*
    - 6 hours post-exposure: 10% decrease*
    - 24 hours post-exposure: 6% decrease*
    - 48 hours post-exposure: 4% decrease
    - 72 hours post-exposure: 7% decrease*

- Myelin from right cerebral hemisphere:

- Protein content: No significant change
- Leucine uptake:
  - 2 hours post-exposure: 24% increase
  - 6 hours post-exposure: 20% increase
  - 24 hours post-exposure: 8% decrease
  - 48 hours post-exposure: 31% decrease*
  - 72 hours post-exposure: 4% increase

*Statistically significant

Author's Conclusions:

- *"The protein content of brain or myelin did not change…whereas the uptake of labeled leucine in the cerebral homogenate was significantly lower than in the control brains at 24 and 48 h after the exposure…A similar decrease in the leucine uptake in the myelin fraction could be detected 48h after the experiment."*
- *"the brain RNA content did not differ from the control range whereas the decrease in the leucine incorporation was accompanied by a decrease in the activity of acid proteinase…the acid proteinase activity remained significantly lower than in the control brain even at the end of the follow-up period."*
- *"…absence of neurological findings in our experiment"*
- *"inhibition of cytochrome oxidase activity which could lead to reduction in the energy-dependent protein metabolism…changes in protein synthesis are accompanied by decreased proteolysis which is compatible with the closely interdependent synthesis and destruction of brain protein."*
- *"…repeated or long-term inhalation of H_2S may lead to toxic manifestations in the brain as the delay of maximal biochemical changes well after the acute episode might allow accumulation of the deleterious effects."*

Review – Assessment:

A. Test animals:	- The number of exposed animals was limited (*i.e.*, 6 per interim sacrifice). Testing guidelines generally recommend use of 10 animals (5 per sex) per exposure group. The number of control animals was severely limited (i.e., 2 per interim sacrifice). +/- Some of the principle characteristics of test animals (*e.g.*, specie, strain, sex, weight variation and source) were reported but the pre-test health status of animals was not indicated. + Body weight variation was in compliance with guideline recommendations. - Only female mice were tested.
B. Exposure conditions:	- The source of the pure H_2S gas was not indicated. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibrium period. - The total animal displacement in the chambers was not reported. - Whether oxygen content, temperature, pressure, humidity and photoperiod were monitored throughout exposure was not reported. + Target concentration of H_2S was confirmed analytically. Authors reported that *"measured H_2S concentration deviated 2% form that of the feed gas"*. + Chamber air was changed 15 times/hour, in compliance with guideline recommendations.
C. Housing/Feeding:	- Details concerning animal housing (*i.e.*, temperature, humidity, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type, and dimensions) was not provided. - Bedding material was not specified.

	- The type and source of feed supplied was not reported. The feeding schedule was also omitted. - Details concerning the water supply were lacking.
D. Equipment:	+ Description of the exposure chamber and gas delivery system was adequate, although a schematic would have been beneficial. +/- The equipment used to measure the gas concentration (*i.e.*, cadmium acetate trapping and iodometric titration) was reported, but method was judged to provide limited sensitivity.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. - Absence of details pertaining to the randomization of test animals and assignment to test groups. + A control groups was included and the control animals were *"exposed in an identical chamber to air"*. - Other than a precursory assessment of behavior, conventional measures of toxicity (*i.e.*, body weights, organ weights, gross pathology) were not included. - Study pre-dated Good Laboratory Practice (GLP) guidelines. - A single exposure concentration was tested, thereby precluding any assessment of dose-responsiveness. - Only two controls were killed at each sacrifice interval. - Whether duplicate analyses of protein content and enzyme activity were conducted was not specified. - Unclear whether technicians and handlers were blinded to exposure conditions.
F. Data collection:	+ The reversibility of changes was monitored up to 72 hours post-exposure. - Individual data were not provided for each test animal, thereby limiting the independent assessment of the findings. - Data from the ten control animals sacrificed following different post-exposure periods were averaged, without proper statistical assessment.
G. Data analysis:	+ Data were statistically analyzed with Student's *t* test. - Confidence intervals were not provided. - SEM was notably large for certain measurements.
H. Interpretations:	- Information pertaining as to why the study investigators choose homogenate from the right hemisphere and myelin samples from the left hemisphere was not provided.

Review - Summary:

Discussion of findings:

Whole-body exposure of female mice to 100ppm H_2S for 2 hours under controlled conditions reportedly produced alterations in protein metabolism in the brain, as evidenced by a reduction in the incorporation of radio-labeled leucine into cerebral protein and myelin, and a decline in acid proteinase activity in the brain. The changes were not accompanied by any shift in the protein content of the brain or myelin. In addition, apart from some agitation during the first 20 minutes of exposure, no clinical signs indicative of neurological distress or dysfunction were observed among the exposed mice.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The study was not directed at determining the health effects *per se* of H_2S exposure, but rather was aimed at possibly contributing to understanding of the mechanism of action of hydrogen sulphide.
- The change in leucine incorporation was not consistent, but rather varied with the time elapsed following exposure. Within the first few hours after exposure, the rate of leucine incorporation into both the brain protein and myelin actually *increased* relative to the corresponding control rate, and decreased only after 24 hours. By 72-hours post-exposure, recovery was evident in both types of tissue. This shift in response renders interpretation of the findings difficult.
- The change in acid proteinase activity was marginal, with less than 10 percent inhibition noted at all interim sacrifice times.

- The changes in leucine incorporation and acid proteinase activity were *not* accompanied by a corresponding change in the protein content or RNA content of the brain or myelin. Thus, it would appear that the changes were not sufficient to cause a significant shift in protein metabolism in the brain.
- The mice remained clinically unaffected during most of the two-hour exposure period, and showed no evidence of neurological dysfunction throughout the 72-hour post-exposure period. Accordingly, the changes in leucine incorporation and acid proteinase activity were without any clinical correlates. Regrettably, no gross or histopathological examination of the brain or myelin tissues was performed to determine if the changes had macroscopic or microscopic correlates.
- In the absence of clinical signs as well as the lack of changes in the protein and RNA content of the brain and myelin, the toxicological significance of the shifts in leucine incorporation and acid proteinase activity is unknown.
- Use of a single exposure concentration only precludes any determination of the dose-responsiveness of the observed changes. Interpretation of the significance of the findings would have been significantly improved through the use of graded exposure levels, consistent with the recommendations of testing guidelines.
- The relevance of the study findings to the human condition is undermined by the use of female mice only. The extrapolation of the findings to humans would have been improved through the use of both sexes of mice.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of only a limited number of test animals (*i.e.*, 6 per sacrifice time).
- Use of the female sex only.
- Use of a single exposure concentration only.
- Failure to include conventional measures of toxicity as part of the overall study design.
- Lack of detail concerning animal housing and husbandry.

Review – Confidence index ranking:

Low to moderate

Document Review

Author:	Haider, SS; Hasan, M; Islam, F	**Internal ID:**	179
Title:	Effect of air pollutant hydrogen sulfide on the levels of total lipids, phospholipids and cholesterol in different regions of the Guinea pig brain		
Year:	1980		
Abstract:	*"Levels of total lipids, phospholipids and cholesterol were estimated in cerebral hemisphere, brain stem, and cerebellum of guinea pigs exposed to hydrogen sulfide (20 ppm) daily for 1 hr for 11 days. Significant lowering in the concentration of total lipids, and phospholipids occurred in cerebral hemisphere and brain stem, but the diminution in cerebellum was insignificant. Levels of cholesterol, however, remained unchanged in different regions of the brain. The rate of lipid-peroxidation was significantly increased in cerebral hemisphere."*		
Objective:	To assess the changes in lipid composition of different regions of the guinea pig brain following subacute H_2S exposure.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 or 20ppm (whole body)	Repeated/ 1hr per day for 11 days	Guinea pig	Not specified	Adult	Male	12 (6 treated; 6 control)	Not specified	NA	20ppm

Observations:

- Biochemical analysis:
  - Total lipids:
    - -Cerebral hemisphere: 34.5% decrease*
    - -Cerebellum: 4.1% decrease
    - -Brain stem: 19.4% decrease*
  - Phospholipids:
    - -Cerebral hemisphere: 11.02% decrease*
    - -Cerebellum: 3.3% decrease
    - -Brain stem: 21.2% decrease*
  - Cholesterol:
    - -Cerebral hemisphere: 1.5% decrease
    - -Cerebellum: 0.4% decrease
    - -Brain stem: 2.6% decrease
  - Lipid peroxidation:
    - -Cerebral hemisphere: 18.4% increase*

- Clinical signs:
  - Fatigue, somnolence, dizziness, itching and eye irritation.

*Statistically significant

Author's Conclusions:

- *"Significant lowering in the concentration of total lipids, and phospholipids occurred in cerebral hemisphere and brain stem, but the diminution in cerebellum was insignificant."*

- *"Levels of cholesterol…remained unchanged in different regions of the brain"*
- *"The rate of lipid-peroxidation was significantly increased in cerebral hemisphere. It is clear that peroxidation of endogenous lipids has been enhanced by H_2S"*
- *"It is clear that the exposure to H_2S causes a depletion of phospholipids and total lipid contents of the cerebral hemisphere and brain stem without affecting the cerebellum".*

Review – Assessment:

A. Test animals:	- The numbers of test animals allocated to each exposure group (*i.e.*, n=6) was limited. Guidelines for acute/subacute toxicity testing generally recommend use of 10 to 20 animals (*i.e.*, 5-10 per sex) per exposure level. +/- The principle characteristics of test animals (*e.g.*, specie, sex and weight) were adequately reported, however, information pertaining to exact age, breed, and pre-test health status was lacking. - Only male guinea pigs were exposed. - The breeding facility from which animals were obtained was not noted. + Exposed animals were adults, which complies with guideline recommendations. + The 20% weight variation of test animals prior to study initiation was consistent with recommended guidelines.
B. Exposure conditions:	- The source of the H_2S was not specified. - Only the nominal exposure concentration was reported (*i.e.*, 20 ppm). No indication was provided that the target exposure concentration was analytically confirmed. +/- A whole body exposure chamber was employed. +/- Whether the concentration in the exposure chamber was equilibrated before or after test animals were placed inside was not stated. This could potentially alter the duration of exposure. Otherwise the exposure period was precisely defined.
C. Housing/Feeding:	- Details concerning animal caging were not available. - Details concerning the housing environment (*e.g.*, temperature, humidity and photoperiod) were not given. + Animals were fed a commercial pellet feed *ad libitum*. Source of the feed was indicated. - Feeding restrictions imposed during exposure were not noted. +/- Water was provided *ad libitum*. No details concerning water supply were given + The temperature, airflow and humidity of the exposure chamber were controlled and values were within recommended guidelines.
D. Equipment:	+/- Basic details concerning the exposure chamber (*e.g.*, volume, temperature, humidity, airflow) and gas delivery system were provided. The suitability and integrity of the equipment was presumed to be adequate.
E. Procedural:	- No acclimation period or pre-test conditions (*i.e.*, quarantine, disease treatment, etc.) were specified. - It was unclear whether test animals were randomly assigned to exposure groups. + A control group of animals was included. - Handling and exposure of the control animals evidently differed from the treated animals (*i.e.*, it would appear that control animals were exposed to room air in cages, outside of the exposure chamber). - Conventional measures of toxicity were largely lacking. Apart from the lipid analyses, only clinical observations were recorded. - Only a single exposure concentration was tested. Guidelines for subacute toxicity testing generally recommended use of graded exposure levels to permit assessment of dose-responsiveness. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	+ The biochemical methods used were referenced thereby permitting reproducibility.

	- Only total cholesterol was measured. It is more meaningful to measure free and esterified cholesterol. - Individual data were not provided for each test animal limiting the independent assessment of the findings.
G. Data analysis:	+ Data were analyzed statistically using Student's *'t'* test
H. Interpretation:	+ The study's primary focus, to assess lipid compositional changes in the brain following H_2S exposure, was appropriately addressed by the experimental design. + The novelty of this study is stated by the author, *"the present investigation is the singular attempt to provide information in this area."* +/- Although some potential confounding variables (*e.g.* age, sex, specie) were considered, the impact of others (*e.g.* housing conditions, handling of animals) was not limited by either the experimental design nor analysis methods. - No analytical confirmation of the target exposure concentration was performed. - The clinical/toxicological significance of the changes in lipid parameters is unknown. - The eye irritation reportedly observed was not confirmed through ophthalmolscopic examination. - Details surrounding the clinical observations were limited. The time of onset, severity and duration of the symptoms were not indicated.

Review - Summary:

Discussion of findings:

Subacute exposure of guinea pigs to 20 ppm of H_2S by inhalation under controlled conditions caused regional-specific reductions in total lipids and phospholipids in brain tissue. Cholesterol levels were unaffected. Evidence of increased lipid peroxidation following H_2S exposure also was presented. Clinical signs of toxicity included fatigue, somnolence, dizziness, itching and eye irritation.

Interpretation of the significance of the study findings should take into consideration the following:

- It would appear that the primary motive of the investigation was not clinically oriented, but rather was driven simply by a lack of information respecting the effects of H_2S on brain lipid composition. No biological rationale for performing the study was provided, other than the statements that *"the metabolic changes caused by [H_2S] and the mechanisms involved therein are not fully understood"*, and *"neurochemical studies of changes in lipid composition of different regions of the guineapig brain following H_2S exposure have so far not been undertaken"*. The authors offered that the *"health hazards due to H_2S are well known"*. Accordingly, the study was not intended nor designed to advance understanding of the health effects *per se* of H_2S, but instead was aimed at exploring the effects of H_2S on brain lipids, possibly to improve understanding of mechanism of action.
- The toxicological and/or clinical significance of the observed changes in brain lipid composition were not established by the study. No attempt was made to identify clinical or pathological correlates of the changes. Other than some limited clinical observations, no routine measures of toxicity were performed. The reported clinical observations were lacking detail with respect to frequency, time of onset, severity and duration, thereby limiting interpretation of significance.
- The reported clinical observations following exposure were consistent with those reported elsewhere in the literature. However, the reported eye irritation was not confirmed by *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination of the eyes.
- The changes in lipid composition were not consistent across all regions of the brain, and varied according to the type of lipid. For example, cholesterol levels were unaffected, and no changes were evident in the cerebellum. The lack of uniformity of response renders the interpretation of the significance of the findings difficult. Not only is interpretation limited by the lack of clinical and histological correlates of the changes, but any explanation of the meaning of the shifts in lipid composition would need to account for the regional differences in response.
- The dose-responsiveness of the observed changes in brain lipid composition could not be determined owing to the use of a single exposure concentration only. Interpretation of the significance of the findings could have been improved through use of graded exposure concentrations, allowing for identification and examination of dose-response patterns.

- The study used male guinea pigs only. Thus, extrapolation of the findings to the human condition is limited owing to the lack of information on the female sex.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of only a limited number of test animals.
- Use of the male sex only.
- Differences in the handling of control and treated animals (*i.e.*, failure to expose the control and treated groups under identical conditions).
- Complete lack of detail concerning animal housing and general lack of detail concerning animal husbandry.
- Use of only a single exposure concentration.
- Failure to analytically confirm the target exposure concentration.

Review – Confidence index ranking:

Low

Document Review

Author:	Hannah, R.S.; Hayden, L.J.; Roth, S.H.	**Internal ID:**	180
Title:	Hydrogen sulfide exposure alters the amino acid content in developing rat CNS.		
Year:	1989		
Abstract:	*"Hydrogen sulfide is a widespread environmental pollutant that may produce severe effects on the developing nervous system. Putative amino acid neurotransmitter levels in the rat cerebrum and cerebellum were determined to evaluate the effects of exposure to hydrogen sulfide during perinatal development. The levels of aspartate, GABA, glutamate, glycine and taurine were quantitated using high-performance liquid chromatography. With the exception of glycine, all of the amino acids examined were affected by the treatment. On day 21 postnatal, which was the last day of the exposure, aspartate, glutamate and GABA in the cerebrum and aspartate and GABA in the cerebellum were significantly depressed. The observed alterations in the amino acid levels during this critical phase of development may have chronically affected the activity of neurotransmitters, their receptor sensitivity or their individual target areas. The consequence of one or a combination of such alterations may lead to behavioral and structural abnormalities."*		
Objective:	To examine the effects of prolonged exposure to low concentrations of H_2S on several putative amino acid neurotransmitters in the developing rat brain using the cerebrum and cerebellum as representative models.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 ppm; 75ppm (whole body)	Repeated/ exposure of dams for 7 hours per day from day 5 postcoitus until day 21 postnatal	Rat	Sprague-Dawley	Not specified.	Female (Pup sex was not specified)	Number of dams was not specified. 48 pups were examined (24 control; 24 exposed)	Timed-pregnant dams	NA	75ppm

Observations:

- Pup weight: No significant difference in either whole body or brain weight.

- Cerebrum amino acid levels (relative to control values):
  - Aspartate:
    - 7 postnatal(PN): 39% lower*
    - 14 PN: 18% lower*
    - 21 PN: 17% lower*
  - GABA:
    - 7 PN: No significant difference
    - 14 PN: ~28% lower*
    - 21 PN: ~31% lower*
  - Glutamate:
    - 7 PN: ~21% lower*
    - 14 PN: ~15% lower*
    - 21 PN: No change

- Glycine:
  - 7 PN: No significant difference
  - 14 PN: No significant difference
  - 21 PN: No significant difference
- Taurine:
  - 7 PN: ~22% higher*
  - 14 PN: ~17% higher*
  - 21 PN: No significant difference

- Cerebellum amino acid levels (relative to control values):
  - Aspartate:
    - 7 PN: ~ 15% higher*
    - 14 PN: ~18% higher*
    - 21 PN: No significant difference
  - GABA:
    - 7 PN: No significant difference
    - 14 PN: 20% lower*
    - 21 PN: 15% lower*
  - Glutamate:
    - 7 PN: No significant difference
    - 14 PN: No significant difference
    - 21 PN: 20% lower*
  - Glycine:
    - 7 PN: No significant difference
    - 14 PN: No significant difference
    - 21 PN: No significant difference
  - Taurine:
    - 7 PN: 25% higher*
    - 14 PN: 17% higher*
    - 21 PN: No significant difference

*Statistically significant

Author's Conclusions:

- *"In summary, the prolonged exposure to a low concentration of H_2S, produced alterations in four of the five amino acids tested in both the cerebellum and cerebral cortex. The consequences of these shifts in the amino acid pool during this critical phase of brain development is as yet unknown but may result in long term behavioral problems."*
- *"The interpretation of these findings in terms of an alteration of available neurotransmitter is very difficult since all of the amino acids examined, with the possible exception of GABA, are probably present in all cell types and may have several non-transmitter roles".*

Review – Assessment:

A. Test animals:	- The number of dams was not specified.

	- The number of pups examined was specified (*i.e.*, 8 per exposure group at each sampling time), but the number of litters from which the pups were secured was not reported. Given that guideline recommendations for developmental toxicity studies generally require the use of 20 dams per exposure group and examination of the corresponding litters that typically consist of 2 to 12 pups (*i.e.*, total of 40 to 240 pups per group), the number of pups examined in the study was judged to be limited. +/- Some of the principle characteristics of test animals (e.g. specie, strain, sex and pre-test health) were reported but the age, weight variation and source of the dams were not defined.
B. Exposure conditions:	- The source of the H_2S gas was not indicated. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibration period. + The exposure device was adequately described. More complete details were found in a companion paper (Laskin and Drew, 1970). + The total animal displacement in exposure chambers reportedly did not exceed 5% of the total volume of the chamber, consistent with guideline recommendations. + Chamber was designed to permit continuous observation of animals. +/- H_2S concentrations, airflow (20 changes per hour), temperature, pressure, humidity and photoperiod were reportedly monitored throughout exposure, but the results of most of these analyses were not reported. Accordingly, it was not possible to verify that the chamber conditions met guideline recommendations, nor to confirm that the target concentration of H_2S was achieved. +/- Concentrations of H_2S were measured at the animal level, but these levels were not reported.
C. Housing/Feeding:	- Details concerning animal housing (*i.e.*, temperature, humidity, photoperiod) were lacking. - It is unclear as to whether the dams and/or pups were housed continuously in the exposure chamber or introduced daily into the chamber during the 7-hour exposure period only. - Details concerning animal caging (*i.e.*, type, dimensions) were not provided. - Bedding and nesting materials were not specified. + Dams were allowed to eat and drink *ad libitum* except during the exposure period, consistent with guideline recommendations. - The type and source of feed supplied was not reported. - Details concerning water supply were lacking.
D. Equipment:	+ Description of the exposure chamber and gas delivery system was adequate. +/- The equipment used to measure the gas concentration and airflow rate were reported but the manner by which humidity, temperature, and oxygen levels were determined was not outlined.
E. Procedural:	- No acclimatization period was reported. - No description of pre-test conditions was provided. +/- Pups were randomly chosen for euthanization but whether dams were randomly assigned to exposure groups was not noted. The method of randomization (*e.g.* table of random numbers, computer generated, *etc.*) was not given. - Details concerning pup selection were lacking. + A control group was included and the control animals were maintained in the same conditions, without H_2S exposure. - Unclear whether technicians and handlers were blinded to exposure conditions. + Some consideration given to avoiding experimental bias vis-à-vis scheduling of animals for euthanization and handling of tissues for analysis (*i.e.*, control and treated pups were sacrificed in parallel). + The period between brain removal and freezing was identical for all animals.

	- Conventional measures of toxicity (*i.e.*, clinical observations, body weights, litter parameters) evidently were not included as part of study. - No indication that study was performed under Good Laboratory Practice (GLP) conditions. - A single exposure concentration only was tested, thereby precluding any examination of dose-responsiveness. Guidelines for developmental toxicity studies generally recommend use of three treatment levels and a corresponding control group.
F. Data collection:	+ The reversibility of changes in amino acid levels was tracked up to PN 21. - Individual data were not provided for each test animal, thereby, limiting the independent assessment of the findings. - Data from the treated animals were expressed only as a percentage of corresponding control values, and captured in figures of limited resolution. No numerical data were presented for the treated pups.
G. Data analysis:	+ Data were statistically analyzed with Student's *t*-test - Confidence intervals were not provided.
H. Interpretations:	- Conclusions were based largely on hypotheses, and were not supported by empirical data. +/- The authors admitted that interpretation of the findings was difficult, and that the significance of the findings in terms of neuronal development was unknown. +/- The role of the amino acids selected for analysis in neurotransmission was described as "putative" only (*i.e.*, the exact role, if any, of the amino acids has not been clearly established).

Review - Summary:

Discussion of findings:

Exposure of pregnant rats to 75ppm H_2S for 7hr/day from day 5 postcoitus until day 21 postnatal resulted in alterations in the levels of selected amino acids in the cerebral and cerebellar regions of the brain in the offspring. The levels of aspartate, GABA, glutamate, and taurine were affected to varying degrees, with differing temporal response patterns during the post-natal period. The level of glycine was unaffected. Several mechanisms to account for the shifts in amino acid levels were postulated. Apart from ruling out the depletion of GABA levels due to loss of Purkinje cells, no empirical data were presented in support of the proposed mechanisms.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- Although the study investigators offered that the changes in amino acid levels might result in long-term behavioral problems, it was admitted that the consequences of the changes were *"as yet unknown"*, and that the interpretation of the significance of the findings *"was very difficult"*. Part of the difficulty relates to the fact that the exact role of the amino acids in neural transmission has not been clearly established. The authors acknowledged that the amino acids have been identified as *"putative"* neurotransmitters only.
- Interpretation of the significance of the findings is also made difficult by the limited number of pups that were examined. The use of only 8 pups per exposure group at each sampling time theoretically could translate to the use of 3 litters per group only. Guideline recommendations for developmental toxicity studies generally require examination of 20 litters. Regrettably, information respecting the number of dams on test and the number of litters from which pups were selected precludes critical interpretation of the study findings. The difficulty in interpretation is compounded by the lack of information concerning the method of selection of pups from the available litters, and the degree to which all litters were represented in the analyses. The study might have been improved by better description of animal handling and disposition (*e.g.*, number of dams/litters involved, assignment of animals to treatment groups), as well as by increasing the number of pups examined. Any association between exposure of the dams to H_2S and changes in the level of amino acids in the CNS of the offspring might have been strengthened by increasing the number of pups examined.
- The significance of the study findings also is confounded by the differences in temporal responses observed for the different amino acids. The shifts in amino acid levels varied during the course of the post-natal period, with no obvious pattern emerging. Depending on the amino acid and the region of the brain examined, the changes in levels relative to control values were progressive (*i.e.*, uniformly increasing or decreasing), shifting (*i.e.*, alternately

increasing or decreasing), recovering or non-existent. The lack of uniformity of response reflects the complexity of neurological development and underscores the difficulty of interpreting the study findings. Adding to the difficulty is the current lack of understanding of the exact role of the amino acids in neurotransmission, and the recognition that other roles exist (*i.e.*, non-neurotransmitter roles) for these compounds.

- Regrettably the study did not include conventional measures of toxicity, including clinical observations of the dams and pups. Although the exposure chamber evidently was designed to permit visual examination of the animals, there was no record of clinical signs. Since the authors postulated that the shifts in amino acid levels *"may result in long term behavioral problems"*, the study might have benefited from detailed observation of the pups during the 21 day post-natal period for signs of neurological abnormalities and/or behavioral deficits. Correlation of such signs with the shifts in amino acid levels would have added to the significance of the findings. In the absence of clinical indications of toxicity, it could be argued that the "functional reserve" of the pup CNS may be sufficient to accommodate the shifts in amino acid levels without functional change and without neurobehavioral deficits. Similarly, observation of the dams might have revealed the nature and extent of signs, if any, of maternal stress due to treatment. Since the levels of amino acids in the CNS of the pups may be related to the circulating levels in the dams, and since stress may alter the circulating levels, careful observation of the dams might have provided some indication as to whether the shifts in amino acid levels represented direct toxicity of H_2S or secondary effects due to maternal stress. By convention, less significance is generally assigned to responses in the fetuses that occur secondary to maternal stress.
- Unfortunately, only a single exposure concentration (*i.e.*, 75 ppm) was tested in the study. This represents a significant departure from guideline recommendations (*i.e.*, 3 treatment levels, plus control) and precludes any assessment of dose-responsiveness. The significance of the study findings would have been strengthened by the use of more treatment levels. With only a single treatment level tested, the results should be considered preliminary in nature. Further study using graded exposure concentrations would be needed to confirm the findings and to assign proper meaning and significance to the results.
- Consideration of the relevance of the findings to the human condition should take into account the nature of the exposure regimen, which extended through much of the gestation period until Day 21 post-partum (at which times the pups would generally be weaned). The equivalent exposure time for humans would extend over several months.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to, undermine the level of confidence that could be assigned to the study findings and conclusions, and in the words of the authors, rendered the interpretation of the results *"very difficult"*. Notable limitations included:

- Lack of information concerning the number of dams and litters followed as part of the study.
- Use of too few pups to provide for meaningful interpretation of the results.
- Lack of information concerning the extent to which litters were represented by the pups selected for examination.
- General lack of details concerning test animals, housing conditions, assignment to treatment groups, and selection methods.
- Lack of numerical data on amino acid levels in the treated pups.
- Use of only a single exposure concentration.
- Failure to include conventional measures of toxicity (notably clinical observations) to permit correlation of the shifts in amino acid levels with clinical indicators of abnormal neurological development.

Review – Confidence index ranking:

Low to moderate

Document Review

Author:	Jappinen, P; Vikka, V; Marttila, O; Haahtela, T	**Internal ID:**	202
Title:	Exposure to hydrogen sulphide and respiratory function		
Year:	1990		
Abstract:	*"A study was carried out to assess possible effects of low concentrations of hydrogen sulphide on respiratory function. The cohort comprised 26 male pulp mill workers (mean age 40.3, range 22-60 years) with a daily exposure to hydrogen sulphide in the workplace, and 10 volunteers, who had asthma (three men, mean age 40.7, range 33 to 50 years, and seven women, mean age 44.1, range 31 to 61 years). The respiratory function of the pulp mill workers was monitored by measuring forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and bronchial responsiveness after at least one day off work and at the end of a workday. Bronchial responsiveness was tested by challenge with histamine. The 10 asthmatic subjects were exposed in laboratory conditions to 2 ppm of hydrogen sulphide for 30 minutes in an exposure chamber. Airway resistance (Raw) and specific airway conductance (SGaw) were assessed by a body plethysmograph, and the ventilatory capacities were measured with a flow volume spirometer. No significant changes in respiratory function or bronchial responsiveness related to exposure to hydrogen sulphide in the pulp mill workers were found. In the asthmatic subjects, Raw was increased by 26.3% and SGaw was decreased by 8.4% on average after exposure to hydrogen sulphide. These changes were not statistically significant. In two subjects, however, changes were greater than 30% in both Raw and SGaw, indicating bronchial obstruction. It is concluded that exposure for a relatively short time to hydrogen sulphide concentrations appreciably higher than those existing in ambient air do not cause noticeable effects on respiratory function."*		
Objective:	To assess the effects of low concentrations of hydrogen sulphide on respiratory function (Note that the study cohort consisted of a group of pulp mill workers who had experienced daily exposures to H_2S prior to assessment **and** a group of volunteers with asthma who had no obvious prior exposures to H_2S. The present review was concerned only with the effects of H_2S on the asthmatic subjects since the worker cohort had experienced "chronic" exposures to H_2S, and therefore, was judged not to be appropriate for the assessment of health effects following "acute" exposures).		

Overall study design:

Exposure level	Exposure frequency / duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
2 ppm (whole body)	Single / 30 minutes	Both	Males: average 40.7 years (range 33-50 yrs). Females: average 44.1 years (range 31-61 yrs).	10. (3 males and 7 females)	All subjects reported to have had bronchial asthma for one to 13 years. All subjects reported to be using medication. Subjects with *"severe"* asthma were excluded from study.	NA	2 ppm

Observations:

Objective responses

- Ventilatory capacity:
  - Pulmonary effects: Forced vital capacity (FVC): No change
  - Forced expiratory volume in one second (FEV_1): No change
  - Forced expiratory flow ($FEF_{25\text{-}75\%}$): No change

- Specific airway resistance (Raw):

- Reported to be increased on average by 26%, but changes were inconsistent between subjects and differences were not statistically significant. In two subjects an increase over 30% was noted.

- Specific airway conductance (SGaw):
  - Reported to be decreased on average by 8.4%, but changes were inconsistent between subjects and differences did not achieve statistical significance. In two subjects an increase over 30% was noted.

Subjective responses:

- Odour detected at start of exposure by all subjects. Odour reported to be unpleasant, but subjects rapidly became accustomed to it.
- Nasal and pharyngeal dryness experienced by all subjects at start of exposure.
- Three of 10 subjects experienced headache following exposure.

Author's Conclusions:

- *"Exposure for a relatively short time to hydrogen sulphide concentrations appreciably higher than those existing in ambient air do not cause noticeable effects on respiratory function".*
- *"When asthmatic subjects were exposed in controlled conditions to 2 ppm of hydrogen sulphide for 30 minutes, no significant changes in respiratory function occurred. The airway resistance increased somewhat after exposure, but it was not reflected in the subjects' clinical condition".*
- *"It is not possible to predict on the basis of this laboratory study what kind of symptoms asthmatic subjects could have at higher concentrations".*
- *"An exposure for a relatively short time to hydrogen sulphide concentrations at least 10 times higher than those usually found in ambient air caused only minor respiratory effects among asthmatic subjects".*
- *"As the material was limited, the results of our study should be considered as preliminary and further studies are warranted".*

Review – Assessment:

A. Subjects:	+/- The test group consisted of 10 subjects. Both sexes were represented; however, the number of male subjects (n = 3) was somewhat limited. The age range of the subjects (31-61 years) was quite diverse. +/- Each subject was reported to have had bronchial asthma for one to 13 years and had been using medication. The severity of asthma was not specifically indicated, but presumably most of the subjects were at least moderately affected given the use of medication and the time period of the study (pre-1990). Severe asthmatics were excluded from testing. +/- The subjects did not serve as their own controls (*i.e.* no control exposures were performed), rather responses were compared to predicted normal values derived from a Finnish general population. + Informed consent was reportedly obtained from each subject. - The subjects' prior exposure to hydrogen sulphide was not well documented, nor was smoking history. It is unknown whether the subjects were recruited from communities in which pulp mills operated, with the possibility of exposure to ambient levels of reduced sulphur compounds, including H_2S.
B. Exposure conditions:	+/- Each subject received a single 30-minute exposure to hydrogen sulphide under controlled conditions in an *"exposure chamber"*. Exposures were *"whole body"*, thereby allowing the odour of hydrogen sulphide to be detected. - The description of the exposure chamber and H_2S delivery system was limited. + The source of the test gas was indicated. +/- Details concerning oxygen flow were provided, but no specifics were given concerning temperature and humidity within the exposure chamber.

	+ The concentration of H_2S within the chamber was monitored continuously using calibrated instrumentation.
C. Equipment:	+ Details concerning the analytical instrumentation were available. - The description of the exposure chamber and H_2S delivery system was limited.
D. Procedural:	+/- Testing was performed simultaneously on two subjects. No indication was provided as to how subjects were assigned and scheduled for testing (*i.e.*, random selection of subjects was not reported). - Control exposures (*i.e.*, 0 ppm) were not performed, rather control values were based on normal predicted values derived from a Finnish general population. +/- Respiratory function was monitored using a body plethysmograph. Measurements were taken before exposure and within 30 minutes post-exposure.
E. Data collection:	+/- Objective measures included forced vital capacity, forced expiratory volume in one second, forced expiratory flow, airway resistance and specific airway conductance. +/- Subjective symptoms were recorded during and immediately following exposure, but the extent of the examination was not indicated. - Individual subject data were not provided.
F. Data analysis:	+/- Data were subjected to statistical analysis involving calculation of means and standard errors of the means, and assessment of differences using the *t* test for paired comparisons. - Analysis involved comparison of *"% predicted"* values for each of the various endpoints of respiratory function measured; however, the basis of these predicted values was not obvious.
G. Interpretation:	- Exposure was "whole body", introducing the possibility that certain of the subjective symptoms as well as objective measures may have reflected the subject's response to the odour of H_2S rather than to its irritant properties. The influence of odour should be considered in the interpretation of results. - Exposure of two subjects simultaneously in the same chamber introduces the possibility of bias in the reporting of subjective symptoms through influence or persuasion. - The reported changes in Raw and SGaw were inconsistent between subjects. Some subjects showed increased airway resistance, while others showed decreased resistance. Certain subjects showed decreased airway conductance, while others showed increased conductance. +/- None of the changes in respiratory function were reported to be statistically significant.

Review - Summary:

Discussion of findings:

Exposure of asthmatic subjects to 2 ppm of hydrogen sulphide for 30 minutes under controlled conditions in an exposure chamber did not cause any obvious impacts on respiratory function. Changes were limited to possible increases in airway resistance and corresponding decreases in airway conductance; however, the responses were highly variable and inconsistent between subjects. No changes were observed in forced vital capacity, forced expiratory flow, or forced expiratory volume in one second. None of the changes in respiratory function achieved statistical significance. It was reported that two of the 10 subjects experienced changes of over 30% in both airway resistance and airway conductance, indicating possible bronchial obstruction. Accordingly, the response among asthmatics appears to be very much subject-dependent and possibly influenced by the severity of the asthmatic condition. The study did not include subjects with severe asthma.

Subjective symptoms were recorded, consistent with those that might be expected during and/or following exposure to H_2S (*i.e.*, odour, nasal and pharyngeal dryness, and/or headache). It is not possible to discern whether or not the subjective symptoms as well as the reported changes in airway resistance and conductance were triggered by the odor of H_2S as opposed to being related to the irritant properties of the gas.

Interpretation of the significance of the study findings should take into consideration the following:

- Testing was performed on a limited number of subjects only. Better definition and understanding of the observed responses likely could have been achieved through use of a larger sample size.
- Testing was not performed on "severe" asthmatics. Responses to H_2S exposure will likely differ depending on the severity of the asthmatic condition.
- Two of the 10 subjects reportedly showed evidence of bronchial obstruction. Unfortunately, the pre-test health status and nature of the asthmatic condition affecting each individual were not provided. Accordingly, it is not possible to segregate the individuals who experienced bronchial obstruction on the basis of a more severe asthmatic condition that could pre-dispose them to respiratory distress caused by H_2S.
- The reported changes in airway resistance (*i.e.*, 26.3% increase on average) and airway conductance (*i.e.*, 8.4% decrease on average) were not statistically significant, and the responses were inconsistent between test subjects. Specifically, 2 of 10 subjects actually showed decreased airway resistance, and 4 of 10 subjects showed increased airway conductance. Use of a larger sample size might have reduced the inter-subject variability.
- None of the test subjects experienced clinical symptoms consistent with respiratory distress, including the two subjects who reportedly showed evidence of bronchial obstruction.
- Control exposures were not performed. Instead changes in respiratory function were assessed on the basis of comparison to "*values from a Finnish general population*". The degree to which these values were representative and were "*matched*" to the test subjects in terms of age, weight, income, education and other demographic factors is unknown.
- The study investigators offered that the findings should be considered preliminary due to the limited number of subjects.

Confidence index:

The confidence index of the study was judged to be moderate. The study was reasonably well designed, conducted and reported. Assessment of the significance and relevance of the study findings and conclusions could have benefited from:

- Use of a larger sample size.
- Performance of control exposures (*i.e.*, exposure of test subjects to both 0 ppm and 2 ppm of H_2S).
- Placement of only a single subject in the exposure chamber.
- Segregation of subjects on the basis of the severity of the asthmatic condition.
- Better description of the exposure chamber and gas delivery system.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Kangas, J. and Savolainen, H.	**Internal ID:**	207
Title:	Urinary thiosulphate as an indicator of exposure to hydrogen sulphide vapour		
Year:	1987		
Abstract:	*"Urinary thiosulphate analyzed as its bromobimane complex by liquid chromatography revealed quantitatively a preceding hydrogen sulphide gas exposure. The highest thiosulphate concentrations were detected 15 h after the exposure. Controls without a contact to sulphides showed low excretion rates of thiosulphate so that exposures clearly below the fatal hydrogen sulphide gas concentrations could be detected. The urinalysis can therefore be used in the occupational health care as well as for forensic ends."*		
Objective:	To determine the time course of urinary excretion of thiosulphate following short-term exposure to low levels of hydrogen sulphide, as a means to assess the suitability of this assay in investigations of both occupational and poisoning cases. (Investigators monitored urinary thiosulphate levels among volunteers following controlled exposures in a chamber and among workers exposed to H_2S in a pelt processing plant. Assessment was limited to the volunteer subjects since the workers were "chronically" exposed.)		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
8ppm; 18ppm; 30ppm (whole body)	Single / 30 to 45 minutes	Not specified	Not specified	Number of test subjects was not specified. A control group was included and consisted of 29 volunteers.	Not specified	NA	NA

Observations:

Objective responses:

- Progressive increase in urinary thiosulphate concentration reported among test subjects up to 15 hours post-exposure. Thereafter, urinary thiosulphate concentration remained low, and was similar to concentrations detected in control subjects.

- Urinary thiosulphate levels were reported to be dependent on both the level of H_2S exposure and the exposure time.

Subjective responses:

- None reported.

**Statistically significant*

Author's Conclusions:

- *"One of the oxidation products of hydrogen sulphide metabolism is thiosulphate, and in this study, we have demonstrated its excretion in urine after exposure to hydrogen sulphide gas at concentrations found in industry. The test is thus suitable for monitoring in occupational health care as well as in the diagnosis of poisoning cases".*
- *"The urinary thiosulphate concentration changed according to the product of gas concentration and exposure time. Therefore, this product accurately predicted the urinary thiosulphate concentration so that the latter can be used as an exposure indicator to hydrogen sulphide gas".*

- *"...we have demonstrated its (thiosulphate) excretion in urine after exposure to hydrogen sulphide gas at concentrations found in industry. The test is thus suitable for monitoring in occupational health care as well as in the diagnosis of poisoning cases."*

Review – Assessment:

A. Subjects:	- Details concerning the test subjects were lacking. No information was provided respecting number, gender, age, health status exposure history or selection criteria. +/- Subjects were described as *"informed volunteers"*, suggesting that informed consent was obtained. +/- Control subjects consisted of 29 *"lumberjacks"* without any prior exposure to hydrogen sulphide. Details concerning age, health status, gender and selection criteria were lacking. - An additional cohort consisted of *"maintenance men"* from a pelt processing plant, but details concerning numbers, age, health status and selection criteria were not supplied.
B. Exposure conditions:	+/- Subjects were reportedly exposed to 8, 18, or 30 ppm of H_2S for 30-45 minutes in a closed chamber. The exact nature of the exposure was not provided, but presumably, it was 'whole body' since no reference to use of a mouthpiece was given. - The exposure concentrations were nominal. No evidence was provided to indicate that the concentration of H_2S in the exposure chamber was analytically determined for any trial. - Details concerning airflow rate, humidity and temperature within the chamber were not supplied. - The duration of exposure was not explicitly stated, but rather was reported to vary from 30 to 45 minutes.
C. Equipment:	- Apart from the statement that exposures were conducted in a *"3 m^3 closed chamber"*, details concerning the exposure equipment were completely lacking. There was no description of the H_2S delivery system.
D. Procedural:	- Details concerning randomization of the test subjects for exposure, if any, were lacking. +/- Control exposures were performed using a group of 29 lumberjacks. (No further details were provided). - The study did not note any type of blinding. - Lumberjacks were used as a control group. Without subject profiles it is unclear whether this control group was appropriate. - Details concerning urine collection schedules and subject compliance were not available, albeit results were corrected for urinary creatinine excretion.
E. Data collection:	+/- Urine samples were collected at regular intervals post-exposure and monitored for the presence of thiosulphate. - The number of samples analyzed was not recorded. - Apart from the collection of urine samples, no other endpoints were monitored. No records of clinical responses during or post-exposure were available.
F. Data analysis:	- Analyses were limited to calculation of the mean and standard deviation for urinary thiosulphate levels among the control subjects only. Comparable analyses were not performed for the test subjects. Graphical representations of the time course of urinary excretion of thiosulphate were supplied for a single test subject and the group of maintenance workers from the pelt processing plant. - Individual subject data and confidence intervals were not provided.
G. Interpretations:	- Lack of detail concerning test subjects and exposure conditions renders interpretation of findings tenuous. Results should be considered preliminary only. +/- Time course of clearance of thiosulphate suggests rapid oxidation and clearance of H_2S following sub-lethal exposures.

Review - Summary:

Discussion of findings:

Exposure of test subjects to 8, 18, or 30 ppm of H_2S under controlled conditions for 30 to 45 minutes was accompanied by the rapid clearance of urinary thiosulphate, with the highest urinary thiosulphate concentration detected 15 hours post-exposure. Thereafter, thiosulphate urine levels resembled control values, suggesting that the absorbed sulphide had been oxidized and depleted within 15 hours after the exposure. The concentration of this metabolite is a product of the exposure concentration and exposure duration. Since the urine concentration peaked at 15 hours post-exposure, this assessment tool has limited application beyond a limited time frame.

Interpretation of the significance of the study findings is limited by the general lack of information concerning the test subjects, exposure conditions, exposure equipment, clinical responses, and actual subject data. The findings suggest that urinary thiosulphate might serve as a diagnostic marker of H_2S exposure under certain conditions (*i.e.*, poisoning cases and occupational settings); however, further study is recommended to discern its usefulness and overall applicability.

The relevance of the findings to the assessment of the health effects of "acute" exposures to H_2S is limited, primarily as a result of the objective of the study. The study was intended to examine the usefulness of urinary thiosulphate as an indicator of H_2S exposure. It was not intended as an investigation of the health effects following H_2S exposure. Nor were any data relevant to the assessment of health effects (*e.g.*, clinical responses) collected.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Lack of detail concerning the test subjects. (Even the number of volunteer subjects was not indicated).
- General lack of detail concerning control subjects.
- Lack of detail concerning exposure conditions.
- Failure to confirm targeted exposure concentrations.
- Lack of information respecting the exposure chamber and gas delivery system.
- Failure to report actual subject data (*i.e.*, data concerning urinary thiosulphate levels were restricted to a single volunteer).
- Lack of detail concerning urine collection schedules and subject compliance.

Review – Confidence index ranking:

Low

Document Review

Author:	Khan, AA; Schuler, M.M.; Prior, MG; Yong, S.; Coppock, RW; Florence, LZ; Lillie, LE	**Internal ID:**	210
Title:	Effects of hydrogen sulfide on lung mitochondrial respiratory chain enzymes in rats		
Year:	1990		
Abstract:	*"Fischer-344 rats were exposed for 4 h to various concentrations of hydrogen sulfide (H_2S) gas and killed either immediately or at 1, 24, or 48 h after exposure. Mitochondrial fractions from lung tissues were assayed for the activities of respiratory chain enzymes. Exposure of rats to a low concentration (10 ppm) of H_2S caused no significant changes in the activities of lung mitochondrial enzymes. However, exposure to sublethal concentrations of H_2S (50-400 ppm) produced marked and highly significant depressions in the activities of cytochrome c oxidase and succinate oxidase complexes of the respiratory chain. The inhibition of cytochrome c oxidase activity in lungs was most severe (>90%) in rats that died from acute exposure to >500 ppm H_2S. In rats exposed to 200 and 400 ppm H_2S, a marked recovery in cytochrome c oxidase activity of lungs was observed at 24 and 48 h postexposure. Studies in vitro with rat lung mitochondria showed that low concentrations of sulfide also caused a similar and selective inhibition of cytochrome c oxidase activity. This effect was reversed upon removal of sulfide either by washing or by oxidation with methemoglobin. The nature of sulfide inhibition of cytochrome c oxidase was noncompetitive with respect to ferrocytochrome c. Because the activities of NADH-cytochrome c reductase and succinate-cytochrome c reductase were not significantly altered by H_2S exposure and in vitro treatments with low concentrations of sulfide, it is concluded that under physiological conditions H_2S would block the respiratory chain primarily by inhibiting cytochrome c oxidase. Such a biochemical impairment would lead to functional (histotoxic) hypoxia in the lung tissues."*		
Objective:	To determine the sensitivity of mitochondrial respiratory chain enzymes in rat lung to H_2S inhalation. Enzyme complexes that were monitored were those catalyzing electron transport between:(i) substrates (NADH/succinate) to cytochrome *c* (NADH/succinate cytochrome *c* reductase); (ii) cytochrome *c* to oxygen (cytochrome *c* oxidase); and (iii) succinate to oxygen (succinate oxidase). (A separate *in vitro* study involving treatment of lung mitochondria with sodium sulfide also was performed, but was not reviewed). Certain details concerning experimental design can be found in companion papers (Lopez *et al.*, 1987 – Study ID 231; Prior *et al.*, 1988 – Study ID 732).		

Overall study design:

Exposure level	Exposure frequency / duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0, **10**, **50**, 200, 400 or 500-700 ppm (whole body)	Single/ 4hrs	Rat	Fischer-344	8-10 weeks	Male	4-6 rats per exposure concentration in main study. Additional groups of treated rats (12/group) were held for post-exposure observation for up to 48 hours as part of an ancillary recovery study.	Not specified. (Rats were sourced from a reputable supplier and presumed to be healthy).	10 ppm	50 ppm

Observations:

- Mortality:
  - **10**,**50**, 200 and 400 ppm: No deaths
  - >500 ppm: Deaths

- Clinical signs:
  - **10**, **50**, 200ppm: No "marked" clinical symptoms of toxicity (No definition of the term "marked" was provided).
  - 400ppm: Transient lethargy
  - >500ppm: No details provided

- Biochemical (Lung mitochondrial enzymes):
  - **10** ppm:
    - Cytochrome *c* oxidase: 5% increase
    - Succinate oxidase: not analyzed
    - Succinate-cytochrome *c* reductase: 3% decrease
    - NADH-cytochrome *c* reductase: 30% increase
  - **50** ppm:
    - Cytochrome *c* oxidase: 15% decrease*
    - Succinate oxidase: No change
    - Succinate-cytochrome *c* reductase: 16% increase
    - NADH-cytochrome *c* reductase: 1% decrease
  - 200 ppm:
    - Cytochrome *c* oxidase: 43% decrease*
    - Succinate oxidase: 40% decrease*
    - Succinate-cytochrome *c* reductase: 5% increase
    - NADH-cytochrome *c* reductase: 6% increase
  - 400 ppm:
    - Cytochrome *c* oxidase: 68% decrease*
    - Succinate oxidase: 63% decrease*
    - Succinate-cytochrome *c* reductase: 10% decrease
    - NADH-cytochrome *c* reductase: 1% decrease
  - >500 ppm:
    - Cytochrome *c* oxidase activity: 92% decrease*
    - No data for remaining enzymes.

- Post-exposure effects:
  - **10** ppm: No effects
  - 200 and 400 ppm: Significant inhibition of cytochrome *c* oxidase at 1 hour post-exposure, with partial recovery at 24 and 48 hours.

*Statistically significant

Author's Conclusions:

- *"Exposure of rats to a low concentration (**10** ppm) of H_2S caused no significant changes in the activities of lung mitochondrial enzymes".*
- *"...exposure to sublethal concentrations of H_2S (**50**-400 ppm) produced marked and highly significant depressions in the activities of cytochrome c oxidase and succinate oxidase complexes of the respiratory chain".*
- *"The results of our study suggest that H_2S intoxication of pulmonary oxidative and related energy metabolic processes could be primarily responsible for cytotoxic and edematogenic changes in respiratory tract tissues observed in rats exposed to high sublethal concentrations of H_2S".*
- *"The results of this study provide strong evidence that lethal concentrations of H_2S do indeed cause almost total inhibition of cytochrome c oxidase".*

Review – Assessment:

A. Test animals:	+/- Number of test animals (4-6 rats per exposure concentration) was judged to be adequate for the purposes of the study, but was lower that that generally recommended by guidelines for acute toxicity testing (*i.e.*, n=10 per exposure level). + Details concerning source, age, and acclimation of test animals were supplied. - Study used male rats only. No explanation provided as to why females were not included. - Body weights and weight variation at initiation were not provided. - It was not reported whether a pre-test health assessment had been conducted.
B. Exposure conditions:	+/- Exposure concentrations were defined, and included concentrations < 100 ppm (*i.e.*, 10 and 50 ppm). Highest concentrations were expressed as a range only (*i.e.*, 500-700 ppm), without explanation for lack of definition. + Duration of exposure was specified (*i.e.*, 4 hours). + Information pertaining to the purity and manufacturer of reagents was provided. + Exposures were 'whole body' and representative of the typical exposure conditions. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure.
C. Housing/Feeding:	+ Details concerning housing environment judged to be adequate (*i.e.*, temperature, humidity and photoperiod controlled and within ranges specified in OECD testing guidelines). - The number of animals grouped in a single chamber was not specified. + Animals fed and watered *ad libitum*. Food and water sources were described.
D. Equipment:	+/- Description of gas delivery system and other equipment provided in companion papers (Lopez *et al.*, 1987: Prior *et al.*,1988).
E. Procedural:	+ Animals acclimated for 2 weeks prior to exposure, consistent with guideline recommendations. + Animals were acclimated to the exposure chamber prior to study initiation. +/- Animals randomly assigned to treatment groups, but the method of randomization (*e.g.*, table of random numbers, computer generated, *etc.*) was not defined. +/- Exposures were conducted in a series of separate experiments owing to a limited number of exposure chambers. + Each experiment was performed with a paired control group. + Controls were also placed within chambers to account for physiological responses associated with this stress. +/- Emphasis directed at measurement of biochemical responses to H_2S exposures. Very little emphasis on conventional measures of toxicity (*e.g.*, body weights, clinical pathology, gross pathology, organ weights). Some attention to clinical signs. + Anesthesia method was assayed for its impact on biochemical results. - Unclear whether technicians and handlers were blinded to exposure conditions. - No indication that the study was performed under Good Laboratory Practice (GLP).
F. Data collection:	- Individual animal data were not supplied. + Biochemical assays employed were referenced.
G. Data analysis:	+/- Description of statistical methods was judged to be adequate

H. Interpretation:	- Study involved male rats only, limiting extrapolation of findings to the general population. +/- Exposures were not performed concurrently, but rather were completed as a series of experiments, each involving a separate control group. +/- Evidence of progressive recovery of cytochrome *c* oxidase activity between 24 and 48 hours post-exposure is of interest in terms of possible latent effects. +/- Changes in enzyme activities were largely confined to high exposure concentrations only (>200 ppm). Effect at 50 ppm was limited to moderate, albeit statistically significant, inhibition of cytochrome *c* oxidase only. - Absence of *"marked"* clinical signs of toxicity at exposure concentrations up to 200 ppm is of unknown significance since no definition of the term *"marked"* was provided.

Review - Summary:

Discussion of findings:

Whole body exposure of male rats to H_2S for 4 hours under controlled conditions produced dose-related changes in the activities of several pulmonary mitochondrial enzyme complexes associated with oxidative metabolism and cellular energy production. Changes were confined largely to high exposure concentrations (*i.e.*, >200 ppm), both in terms of the number of enzyme complexes affected and the severity of the effects. No changes were observed at an exposure concentration of 10 ppm. At 50 ppm, effects were limited to a reduction in cytochrome *c* oxidase activity only. At higher concentrations, marked inhibition of cytochrome *c* oxidase and succinate oxidase were reported. Clinical evidence of toxicity was confined to the high exposure concentrations, and ranged from transient lethargy among rats exposed to 400 ppm to deaths at 500 to 700 ppm. Exposure of rats to 10, 50 or 200 ppm caused no marked clinical signs of toxicity. The NOEL for the study was judged to be 10 ppm. The corresponding LOEL was judged to be 50 ppm on the basis of the reported inhibition of cytochrome *c* oxidase.

Interpretation of the significance of the study findings should take into consideration the following:

- The inhibition of cytochrome *c* oxidase following exposure to H_2S is well documented. The findings from this study add to the understanding of the inhibition in terms of dose-responsiveness, recovery, and clinical correlates in a tissue directly involved in respiratory activity (*i.e.*, the lungs).
- The dose-responsiveness of the inhibition of cytochrome *c* oxidase was obvious. No inhibition of enzyme activity was observed at 10 ppm of H_2S. Moderate inhibition was noted at 50 ppm , which became progressively greater with increasing exposure concentration. Inhibition was severe at 500 to 700 ppm.
- The inhibition of cytochrome *c* oxidase observed at 50 ppm (*i.e.*, 15%) was comparable to the variability in enzyme activity observed among the various control groups of rats used in the study (*i.e.*, 18%).
- At the higher exposure concentrations (*i.e.*, >200 ppm), correlation between the inhibition of cytochrome *c* oxidase and clinical signs of intoxication was evident. However, despite moderate to marked inhibition of enzyme activity at 50 to 200 ppm, no obvious clinical evidence of toxicity was presented. However, at these lower exposure concentrations, interpretation of the degree of correlation was hindered by the equivocal nature of the statement that *"no marked clinical symptoms of toxicity"* were observed.
- Recovery of cytochrome *c* reductase activity post-exposure was evident, signifying the reversibility of the response. Partial recovery from inhibition was apparent within one-hour post-exposure.
- The study was performed using male rats only. The changes in respiratory enzyme activities in response to H_2S exposure were not examined in the female sex.
- Rats are obligate nasal breathers. Accordingly, penetration of H_2S into the lungs may be attenuated by removal of the gas as it is swept across the nasal passages, thereby reducing the amount of H_2S available to interact with the respiratory enzymes found within the lung mitochondria. Extrapolation of the findings to other species would need to consider differences in breathing patterns as well as differences in ventilation rates and other measures of respiratory function.

Confidence index:

The confidence index of the study was judged to be moderate. Study design, conduct and reporting were considered to be adequate for the intended purpose of the study. Interpretation of the significance and relevance of the study findings could have been improved through:

- Use of larger group sizes of animals. The use of as few as 4 rats per exposure concentration undermines the level of confidence that can be assigned to the findings and conclusions, and does not satisfy guideline recommendations.
- Use of both sexes of animals. Use of the male sex only limits the ease with which the findings can be extrapolated to the human condition.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Khan, KK; Yong, S; Prior, MG; Lillie, LE	**Internal ID:**	211
Title:	Cytotoxic effects of hydrogen sulfide on pulmonary alveolar macrophages in rats		
Year:	1991		
Abstract:	*"Respiratory rates (basal and zymosan-stimulated) and cell viability were monitored in pulmonary alveolar macrophages (PAM) from rats exposed to 0, 70, 280, and 560 mg/m^3 (0, 50, 200, and 400 ppm) hydrogen sulfide (H_2S) gas for 4 h. Zymosan-stimulated respiratory rates were markedly reduced in PAM collected from rats exposed to 280 and 560 mg/m^3 H_2S; however, their basal respiratory rates were not affected. Significant decrease in cell viability was also observed in samples from 560 mg/m^3 H_2S-treated rats, but it remained high and unchanged in other treatments. In vitro incubation of PAM from control rats with sulfide (a precursor of H_2S) and its two oxidation products, sulfite and sulfate, showed that sulfide was markedly more inhibitory to both respiratory rates than sulfite or sulfate. These treatments did not affect cell viability."*		
Objective:	To investigate the effects of H_2S exposure on baseline and particle-induced (zymosan-stimulated) respiratory rates and cell viability in pulmonary alveolar macrophages (PAM) obtained from rats exposed for 4 hours to various concentrations of H_2S gas. (Note that the study was part of a series of investigations of the effects of H_2S exposures on the cellular architecture, oxidative metabolism and integrity of the respiratory tract of male rats. Companion papers covering this series of studies include: Lopez *et al.*, 1987 – Study ID 231; Lopez *et al.*, 1988 – Study ID 233; Prior *et al.*, 1988 – Study ID 732; and Khan *et al.*, 1990 – Study ID 210). In addition to the inhalation exposures to H_2S, the study also included *in vitro* exposures of lung pulmonary alveolar macrophages to sodium sulfide, sodium sulfite and sodium sulfate solutions. These *in vitro* studies were not examined as part of the current review.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; **50ppm**; 200ppm; 400 ppm (whole body exposure)	Single/ 4 hours. (PAM cells were harvested immediately post-exposure).	Rat	Fischer-344	10 weeks	Male	Four groups consisting of 6 rats each.	Not specified. (Rats were obtained from a reputable supplier and presumably were healthy at study initiation).	50 ppm	200 ppm

Observations:

- Mortality and clinical signs: None stated.

- Pulmonary alveolar macrophage (PAM) cellularity:
  - **50**ppm: 1% increase
  - 200ppm: 12% increase
  - 400ppm: 95% increase

- PAM viability:
  - **50**ppm: 3% decrease
  - 200ppm: 4% decrease
  - 400ppm: 18% decrease*

  - Note that exposure to the chamber conditions alone was reported to cause a 5-10% reduction in PAM viability.

- PAM respiratory rate:
  - Basal respiratory rate: No change
  - Zymosan-stimulated respiratory rate:
    - **50**ppm: No change
    - 200ppm: 60% decrease
    - 400ppm: 65% decrease.

*Statistically significant

Author's Conclusions:

- *"Exposure of rats for 4h to various concentrations of H_2S did not affect the number of recovered PAM; however, cell viability was significantly…reduced in the 560mg/m3 H_2S exposure group."*
- *"PAM obtained from rats exposed to H_2S showed no change in the basal respiratory rate, but their zymosan-induced stimulation of respiratory rates was completely abolished in PAM obtained from rats exposed to 280 and 560mg/m3 H_2S."*
- *"These differences in sensitivity of basal and zymosan-stimulated respiratory rates to H_2S (sulfide) could be attributed to (1) specific affinities and natures of H_2S effects on key oxidative enzymes and respiratory components and (2) different intracellular and intramembrane locations of associated respiratory components."*
- *"…H_2S-related dysfunction in the PAM phagocytic process is primarily caused by impairment in related respiratory process".*
- *"A lack of inhibition of the basal respiratory rate of PAM isolated from exposed rats can be attributed to the reversible nature of cytochrome* c *oxidase inhibition by H_2S".*
- *"Our data would suggest that at the exposure concentrations used, the basal respiratory process was either not significantly impaired or progressively reactivated due to possible detoxification of absorbed H_2S".*
- *"More research is needed to ascertain the sites at which H_2S or its oxidation products interfere with PAM's function-specific physiological and biochemical processes".*

Review – Assessment:

A. Test animals:	+/- Number of test animals (6 per exposure concentration) judged to be adequate for the purposes of the study; however, the number was lower than that generally recommended by testing guidelines for acute toxicity studies (n=10 per exposure group). +/- Age, breed and source of test animals were specified. +/- Health status of the test animals at study initiation was not specified, but rats were sourced from a reputable supplier and presumed to be healthy. - Male rats only were used. No explanation was given as to why females were excluded. - Body weight and weight variation of test animals at study initiation were not specified.

B. Exposure conditions:	+ Source of hydrogen sulphide gas was provided. +/- Graded exposure concentrations were used. +/- Duration of exposure (*i.e.*, 4 hours) was specified. +/- Whole body inhalation exposure was studied, thereby mimicking the human condition vis-à-vis exposure of the general population. + Details concerning airflow rates and chamber air pressure were supplied. Flow of H_2S and air was reportedly regulated to maintain target concentrations of the gas in the exposure chamber.
C. Housing/Feeding:	+/- Rats were housed individually. - Details concerning type of cages and bedding materials was not supplied. + Housing environment was controlled, with temperature, humidity and photoperiod regulated and maintained in range recommended in testing guidelines. +/- Feed and water provided *ad libitum*. No indication as to whether or not feed was withheld overnight prior to exposure. +/- Source of feed was indicated (*i.e.*, commercial-grade ration). - Source of water was not indicated.
D. Equipment:	+/- Description of gas delivery system and other exposure-related equipment was provided in companion papers.
E. Procedural:	+ Test animals were acclimated for two weeks prior to exposure, consistent with guideline recommendations. +/- Animals were randomly assigned to test groups, but method of randomization was not reported (*e.g.*, table of random numbers, computer generated, etc). + Control animals were included in the study. + Controls were also placed within chambers to account for physiological responses associated with stress. + All rats were acclimated to the exposure chamber prior to study initiation. +/- Animals were sacrificed immediately following exposure, with no post-exposure observation period. - No details were provided as to whether animals were sacrificed in a random or scheduled order in order to minimize any bias in post-exposure analyses. - Unclear whether technicians and handlers were blinded to exposure conditions. +/- Endpoints examined were consistent with the objectives of the study. However, little or no attention was given to conventional measures of toxicity (*e.g.*, clinical observations, body weights, clinical pathology, gross pathology, organ weights). + Anesthesia method had been previously assayed for its impact on biochemical results. + Graded exposure concentrations were tested. - No indication that the study was performed under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- Individual animal data were not reported. - Cell viability was determined by the trypan blue dye exclusion method. Although it is an acceptable technique, it yields only crude measurements.
G. Data analysis:	+/- Test findings were presented as means and standard deviations of the means. +/- Statistical methods were indicated.
H. Interpretation:	- Clinical significance of changes in PAM parameters is largely unknown. - Correlation between changes in viability and respiratory rate of pulmonary alveolar macrophages and clinical signs of H_2S intoxication has not been established. - Study used male rats only. - Changes in PAM parameters were confined to high exposure concentrations (*i.e.*, >200 ppm).

Review - Summary:

Discussion of findings:

Whole body exposure of male rats to graded concentrations of H_2S for 4 hours under controlled conditions in an exposure chamber resulted in dose-related changes in the viability and respiratory rates of pulmonary alveolar macrophages. Responses were confined to the high exposure concentrations (*i.e.*, 200 and 400 ppm). No changes in PAM parameters were noted among the rats exposed to 50 ppm.

Interpretation of the significance of the study findings should take into consideration the following:

- No changes in the viability or respiratory rates of the pulmonary alveolar macrophages were caused by exposure to 50 ppm of H_2S. Changes were only observed at the higher concentrations.
- No reference was found to clinical signs of toxicity among any of the groups of exposed rats. Accordingly, the observed changes in the viability and function of the pulmonary alveolar macrophages at the cellular level did not appear to be accompanied by any clinical expressions of intoxication.
- Rats are obligate nasal breathers. Evidence suggests that the penetration of H_2S to the alveolar regions of the lung in rats is reduced as the gas is swept across the nasal passages. Accordingly, use of the rat as a model could misrepresent the effects of H_2S exposure on the pulmonary alveolar macrophages of other species, such as humans, that breathe through both the mouth and nose, thereby allowing greater opportunity for the gas to reach the alveolar spaces.
- The study was performed using male rats only. Effects on the female sex were not examined.

Confidence index:

The overall design and conduct of the study were judged to be adequate for the purposes of the investigation. Interpretation of the significance of the study findings might have been improved by:

- Use of larger animal group size. The use of only 6 rats per exposure concentration was somewhat limited. Most testing guidelines recommended the use of 10 animals (5/sex) per exposure level.
- Use of both sexes of rats. The use of the male sex only limits the usefulness of the study vis-à-vis extrapolation of the findings to the human condition.
- Greater attention to conventional measures of toxicity, including routine clinical observations. In the absence of clinical measures of toxicity, it is difficult to gauge the clinical significance and relevance of the study findings.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Kilburn, KH	**Internal ID:**	216
Title:	Exposure to reduced sulfur gases impairs neurobehavioral function.		
Year:	1997		
Abstract:	*"Background: In the 19th century, deaths from acute exposure to hydrogen sulfide (H_2S) portended permanent brain injury from nonlethal doses. The neurobehavioral effects of H_2S exposures lasting from moments to years were compared in 16 subjects, 2 years to 22 years afterward.* *Methods: Neurophysiologic and psychologic tests were used to appraise mood status and frequencies of 35 symptoms. Functions and frequencies, described as percent predicted adjusted for age, sex, educational achievement, and other factors, were compared with those in an unexposed population.* *Results: Frequencies were elevated for 31 of 33 symptoms. Balance was impaired (246% predicted with eyes closed, 159% predicted with eyes open), and simple and choice reaction times were prolonged (151% and 130% predicted, respectively). Visual fields performance was decreased to 72% predicted (right) and 55% predicted (left), color discrimination was abnormal, and hearing was decreased. Psychologic domains showed cognitive disability, reduced perceptual motor speed, impaired verbal recall and remote memory, and abnormal mood status."*		
Objective :	To assess H_2S-related impairment of neuropsychologic function among human subjects, 2 years to 22 years after exposure, using a battery of neurophysiologic and psychologic tests. Note that three exposure cohorts were defined on the basis of the duration of H_2S exposure These cohorts were labeled the "minutes group", the "hours group" and the "years group". A control or "referent" group also was included. The exposure concentrations for group (a), the minutes group, and the exposure duration of group (c), the years group, were outside of the range of interest for the present assessment. Accordingly, emphasis was directed at the findings from group (b), the hours group.		

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
(a) 328ppm to 1000ppm (b) 1ppm to 50ppm (c) Unknown (d) 0ppm (referent)	(a) 5 to 10 min (b) 1 to 24 hours (c) 11 to 22 years (d) None	(a) Male (b) Both (c) Both (d) Both	(a) 27 to 68 years (b) 21 to 62 years (c) 42 to 63 years (d) Unknown	(a) 5 (b) 6 (c) 5 (d) 353	*"All patients had complete physical and clinical screening"*	NA	NA

Observations:

Note that unless indicated otherwise, the following observations relate only to Group (b). The "scores" reflect the study investigator's interpretation of the findings. Independent assessment was precluded by a lack of information concerning typical or referent values. Considerable variability in scores existed between subjects.

Objective responses:

- Neurophysiologic tests:
    - Balance (eyes open): Abnormal (3 of 6 subjects)
    - Balance (eyes closed): Abnormal (5 of 6 subjects)
    - Simple reaction time: Abnormal (5 of 6 subjects)
    - Choice reaction time: Abnormal (4 of 6 subjects)
    - Blink reflex latency: Abnormal (4of 6 subjects)
    - Color discrimination: Abnormal (4 of subjects)
    - Visual fields score): Abnormal (3of 6 subjects)
    - Visual performance (Right): Abnormal (number of subjects affected not reported)

- Visual performance (Left): Abnormal (number of subjects affected not reported)
- Grip Strength: Abnormal (number of subjects affected not reported)
- Hearing: Abnormal (number of subjects affected not reported)

- Cognitive:
  - Culture fair (non-verbal nonarithmetical intelligence): Stated to be normal (2 of 6 subjects affected)
  - Block design (constructional, interpretative attention and integrative capacity): Stated to be normal (2 of 6 subjects affected)
  - Vocabulary: Normal/Abnormal (inconsistent reporting of findings)

- Perceptual motor speed:
  - Peg placement: Abnormal (2 of 6 subjects affected)
  - Trails A score (dexterity, coordination, decision making and peripheral sensation and discrimination): Stated to be abnormal (0 of 6 subjects affected)
  - Trails B score: Stated to be abnormal (3 of 6 subjects affected)

- Verbal recall immediate: Normal (0 of 6 subjects affected)
- Verbal recall delayed: Abnormal (4 of 6 subjects affected)

- Remote memory:
  - Information: Stated to be normal (2 of 6 subjects affected)
  - Picture completion: Normal (1 of 6 subjects affected)
  - Similarities: Normal (1 of 6 subjects affected)

Subjective responses: (NB: observations are not specific to the minute, hour or year exposure groups)

- Symptom frequency
  - Mucous irritation: Increased*
  - Chest symptoms: Increased*
  - Sleep: Increased*
  - Memory: Increased*
  - Balance: Increased*
  - Mood: Increased*
  - Gastrointestinal symptoms: Increased*
  - Limbic functions: Increased*
- Major symptoms at time of exposure (Group b):
  - Loss of strength (syncope), headache, excessive fatigue, pain in chest and back, unconsciousness and/or awareness of odor
  - Symptoms varied by subject

*Statistically significant

Author's Conclusions:

- *"Permanent neurobehavioral impairment was apparent in all 16 subjects who were tested months to years after momentary long-term exposure to reduced sulfur gases"*
- *"It is unlikely that the symptoms found in this study could be attributed to other causes, since extensive questionnaire data showed that head injury or other*

causes of unconsciousness and neurologic and psychiatric disease were rare in exposed subjects"
- *"A few hours of occupational H_2S exposure without unconsciousness or respiratory distress permanently impaired neurobehavioral function."*
- *"CNS hypoxia due to respiratory failure was not present in 11 of the 16 patients and thus could not explain the findings"*
- *"Consistent sensitive observations of "natural experiments" as in this study focus attention on the danger of H_2S exposure, but they are rarely considered definite proof. The doubts aroused should stimulate epidemiologic studies".*
- *"Well beyond calling for epidemiologic studies, these findings imply that neighborhoods near refineries and other industrial sites where H_2S is released deliberately or inadvertently are unsafe".*

Review – Assessment:

A. Subjects:	- The number of subjects per exposed cohort group (n = 5 to 6) was very limited. This was compounded by the fact that exposure conditions varied markedly between subjects of the same exposure group. + Subject characteristics were adequately described (*i.e.*, age, sex, education level, occupation). +/- Age range of subjects was large. +/- Patients had complete physical and clinical screening neurologic examinations, but details were not supplied. + Informed consent was given. - Subjects were likely previously exposed to H_2S due to the nature of their occupations. - Occupations varied, but each presented the possibility of multiple chemical exposures (*e.g.*, tank cleaner, process operator in chemical plant). - Twelve, of the total of 16 subjects, were reportedly plaintiffs in lawsuits introducing the possibility of selection and subject bias.
B. Exposure conditions:	- The exposure estimate for the "hours" group was 1-10ppm, but exposure estimates for selected members of this group were notably higher than this range. (*e.g.*, Subjects 1 and 2 were likely exposed to 25 to 50ppm H_2S). - As noted by the author *"exposures were estimated"*. No actual measures of exposure were available. - Exposure estimates were based largely on threshold for odor fatigue, which was set at 1 to 10 ppm. +/- Duration of exposure was average within each group (*i.e.*, minutes-group, hour-group and year-group). However, actual exposure duration was provided for each subject, and ranged from 1 to 24 hours for Group (b).. - Duration of exposure evidently was estimated based on recall of events that occurred 1.7 to 6.3 years earlier.
C. Equipment:	NA
D. Procedural:	+ Controls *"were picked at random from voter registration rolls"*. +/- The author states that controls were recruited from regions where there was *"no evidence of chemical contamination of air or water"*; however, the chemicals that were screened were not noted. +/- Control subjects were matched for sex, age, and years of education, but other confounders (*e.g.*, intelligence quotient, parental intelligence, socio-economic status) were not addressed. + The protocol was approved by the Human studies Research Committee of the University of Southern California School of Medicine. - *"the H_2S exposure status of the 16 subjects was known to the author-tester"* (*i.e.*, the investigators were not blinded to the exposure status of the subjects). - The frequency of 35 complaints was assessed by a *self-administered* questionnaire on an 11-point scale. + All patients had complete physical and clinical screening neurologic examinations. - Evaluations were completed 1.7 to 6.3 years following exposure.

E. Data collection:	+/- Individual data were provided for selected parameters only. - For certain parameters (*i.e.*, complaint frequencies), results were expressed on the basis of group mean values combined across all exposure groups. - *"Intervals from onset of exposure to evaluation varied considerably but were longer in those with years of duration"*. The intervals were between 1.7 and 6.3 years. +/- The evaluation methods employed were, according to the author, extremely sensitive. The validity and sensitivity of the methods were not confirmed as part of the review.
F. Data analysis:	+ Adjusted for age, sex, and educational achievement. - Certain analyses did not distinguish between cohorts, rather findings were combined across all groups and compared to the referent group without regard to differences in exposure concentrations and exposure duration. - Statistical analyses of neurobehavioral performance scores evidently involved comparison of individual exposed subject *"scores"* against group mean referent values. The basis for this type of comparison could not be determined.
G. Interpretations:	+/- The author stated that *"with the exception of patients 12 and 15, these individuals lacked the educational background to understand manipulating test results"* and that *"evidence of malingering was absent in psychologic testing and questionnaire responses."* The basis of each of these statements could not be determined. + Kilburn considered the effects of some potential confounding variables as evidenced by the following statement: *"Extensive questionnaire inquiry for medical, neurologic, and psychiatric diseases showed no causative factors at home and no occupational exposures to chemical or other neurotoxins. Nor did these patients' impairment patterns resemble those of posttraumatic stress syndrome, minimal head injury or chronic fatigue syndrome."* - *"unquestionably, refineries leak carbon monoxide and reduced sulfur gases such as carbon bisulfide and mercaptans, which accompany H_2S and have the same toxic mechanism and are therefore operationally inseparable....to tentatively attribute human impairment to the effects of reduced sulfur gases seems plausible."* This statement seems justified, but the author's reasoning for solely attributing observed effects to H_2S was not. Statements such as, *"Although alternative explanations (e.g., exposures to other chemicals, including drugs and ethanol and neurologic/psychiatric disease) were considered in each individual, the composite experience makes neurobehavioral toxicity from H_2S most probable"*, were not supported by detailed analysis. - At least some of the subjects were plaintiffs in lawsuits, introducing the very real possibility of subject bias. - There was considerable uncertainty surrounding the exposure concentrations of hydrogen sulphide experienced by the subjects. Exposure concentrations were estimated based on symptoms experienced by subjects (*i.e.*, largely the detection of odour), and not on any measurement of H_2S. - Interpretation of many findings was precluded by lack of detail concerning the performance characteristics and test scores of the referent group. In particular, the individual subject data for neurobehavioral performance from each of the exposed cohorts could not be referenced to the control group owing to a lack of even group mean absolute scores for the referent subjects. - Inconsistent reporting of findings was evident for certain parameters (*e.g.*, vocabulary).

Review - Summary:

Discussion of findings:

Short-term exposure to reportedly low levels of hydrogen sulphide (<50 ppm) among six subjects (*i.e.*, the *"hours group"*) was claimed by the author to have caused decrements in neurobehavioral performance and neurophysiologic function as measured by a series of neurophysiologic and psychologic tests. Decrements included delayed reaction time, reduced grip strength, impaired balance, reduced perceptual motor speed, impaired hearing, increased depression, altered moods, and increased complaints of symptoms affecting sleep, memory, gastrointestinal function and limbic functions.

Interpretation of the meaning and relevance of the findings should take into consideration the following:

- This study aimed to assess the neurophysiologic and neurobehavioral effects following "acute" exposure to low levels of H_2S among human subjects. As such, it was relevant to the present review.
- Exposure estimates were questionable. The evidence indicates that exposure concentrations may have been significantly higher than those reported (*i.e.*, 1 to 50 ppm). For example, one of the 6 subjects from the "hours" group was rendered unconscious by the exposure. Similarly, the exposure estimates were based on the threshold for olfactory fatigue, which the author reported to be between 1 and 10 ppm. Most estimates of the threshold for olfactory paralysis for H_2S range between 150 and 200 ppm. In all cases, exposure concentrations represented *estimates* only, and not measured values.
- The 6 subjects of the "hours" group were all employed in occupations (*e.g.*, oil refinery bargeman; chemical process technicians, tank cleaner, and chicken feed deliverer) associated with chronic H_2S exposure. Moreover, these occupations presented the very real possibility of exposure to other chemicals, thereby seriously confounding interpretation of the findings.
- Only a very limited number of test subjects were included within each exposure 'cohort'.
- Recall bias may have confounded the results. There was a considerable lag period between time of exposure and evaluation of the exposed subjects (*i.e.*, from 1.7 to 6.3 years for "hours" group).
- Twelve of the sixteen subjects were plaintiffs in lawsuits, introducing the very real possibility of subject bias.
- Certain parameters (*i.e.*, symptom complaints, profile of moods score) were assessed on the basis of a *self-administered* questionnaire only, without independent confirmation.
- For most test parameters, considerable variability existed in the performance 'scores' of the individual exposed subjects. For several parameters, the differences in neurobehavioral performance scores between the exposed subjects and the referent group achieved statistical significance in only one or two of the 6 subjects comprising Group (b). For other parameters, scores were divided between 'normal' and 'abnormal'.
- The author 's conclusion that *"Although alternative explanations (e.g., exposures to other chemicals, including drugs and ethanol and neurologic/psychiatric disease were considered in each individual, the composite experience makes neurobehavioral toxicity from H_2S most probable"*, was not supported by empirical evidence.
- Statistical analyses compared individual "scores" against group mean referent values, which was judged to be inappropriate.
- The summary findings reported in Tables 2 and 4 did not distinguish between cohorts. Rather, the findings were "pooled" from all subjects, regardless of the concentration or duration of exposure, and compared to referent groups. This failure to segregate test findings between various exposure cohorts hinders any meaningful assessment of concentration-response relationships.
- The reporting of certain findings was inconsistent. For example, according to the text, *"The 6 subjects with hours of exposure did not show abnormalities on....vocabulary"*; however, in Table 3, 50% of the subjects showed significant changes in vocabulary.

Confidence index:

Confidence in the study findings and conclusions was marginal. Confidence was undermined by a number of serious weaknesses in study design, conduct and reporting that rendered interpretation of the findings tenuous. The weaknesses included:

- Very limited number of test subjects. Each exposure "cohort" was restricted to 5 or 6 subjects only.
- Lack of reliable exposure estimates. Estimates were based on recall of events occurring up to 6 years following exposure, and involved "crude' indicators only, notably smell and olfactory fatigue. Measurement of H_2S was lacking in all cases. In some cases, exposure concentrations were very likely underestimated.
- Possible selection bias in choice of test subjects. Seventy-five percent of the subjects were plaintiffs in class action lawsuits surrounding the alleged exposure incidents.
- Possible subject bias in the assessment of certain parameters. Symptom complaints and "profile of moods" scores were based on self-administered questionnaires only.

- Inappropriate "pooling" and reporting of findings across all exposure cohorts, thereby precluding assessment of dose-responsiveness.
- Failure to provide "referent" data to allow for meaningful independent analysis of findings.
- Failure to implement routine "blinding" procedures to minimize experimental bias.
- Failure to adequately account for exposures to other chemicals as a potential confounding variable. Each of the test subjects was involved in an occupation presenting the possibility of chemical exposures.
- Failure to account for confounding variables other than age, sex, height and level of education as part of the assessment of neurobehavioral function. Other obvious factors that should have been considered in the evaluation of neuropsychologic function include socio-economic status and intelligence quotient.

Review – Confidence index ranking:

Low

Document Review

Author:	Kilburn, KH	**Internal ID:**	217
Title:	Evaluating health effects from exposure to hydrogen sulfide: central nervous system dysfunction.		
Year:	1999		
Abstract:	*"The objective is to consider evidence for effects of hydrogen sulfide (H_2S) on the human central nervous system (CNS), pulmonary tract, heart and other systems. Ocular and olfactory function effects will be considered with the CNS. Most effects are due to poisoning of cytochrome oxidase, a respiratory enzyme. Direct effects will be distinguished from those due to secondary hypoxia. There is little evidence for effects on other organs, although there is little data found in reviews in 1992, 1994 and 1997. The effects of other reduced sulfur gases, particularly mercaptans and carbon oxide sulfide have been included but not those from sublethal doses. The question that needs to be answered is can any rotten egg gas be tolerated without persistent harm? The dose-response relationship proposed from H_2S predicts adverse effects at low levels. These are based on four groups of exposed patients arranged in order of numbers of neurobehavioral abnormalities. Best estimates of exposure levels were assigned to these groups to compare effects with the magnitudes and durations of exposure. There were 4.0 abnormalities at H_2S levels exposure below 1 ppm. Exposures in the 1-5 ppm range were associated with eight abnormalities. Levels between 20 and 40 ppm were associated with 12 abnormalities. People exposed to above 40 ppm had 18 abnormalities. The patterns of abnormality were plausible and patterns were consistent as doses increased. Absence of effects of duration of exposure suggested that H_2S injury is all or none. Effects on the heart need further investigation and separation from those of carbon disulfide (CS_2), possible effects on the lung include asthma and pulmonary edema."*		
Objective:	To consider evidence for effects of hydrogen sulfide (H_2S) on the human central nervous system (CNS), pulmonary tract, heart and other systems. Emphasis was directed at the study of the neurophysiological and neurobehioral effects of H_2S exposure. [Note: The study examined four cohorts: "Group 1" consisted of 24 people exposed to low concentrations of H_2S (*i.e.*, 0.1 to 5 ppm) for several months owing to migration of gas deposits found under foundations and crawl spaces of homes; "Group 2" consisted of 13 workers and 22 residents living downwind from a petroleum refinery with a desulfurization unit; "Group 3" consisted of 44 residents exposed to H_2S for up to one week as a result of a refinery explosion and fire; and, "Group 4" consisted of 16 test subjects exposed to varying concentrations of H_2S (*i.e.*, 0.3 to 10,000 ppm) for varying periods of time. Only the findings from Group 3 were subjected to detailed review since the exposure conditions for the remaining cohorts did not satisfy the "inclusion" criteria for the present assessment.].		

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health status	NOAEL	LOAEL
Exposure estimates for *"Group 3"* were inconsistently reported. Methods section: 1-24 ppm Results section: 20-40ppm	Single and repeated exposures during the course of one week following a refinery explosion and fire.	Not specified	"Adults" (Age varied from 37 to 47 yrs)	44 – 48 individuals ("Group 3")[1]	Not specified	NA	NA

[1] Note that "Group 3" consisted of two sub-cohorts, one consisting of 24 Afro-Americans, and the other consisting of 20 Hispanics.

Observations:

Objective responses:

- Group 3: (*1-24ppm*/ 20-40ppm)*
    - Balance:
        -Eyes open: Normal
        -Eyes closed: Normal/Abnormal (inconsistent findings between sub-cohorts)
    - Reaction time:
        -Simple: Abnormal
        -Choice: Normal/Abnormal (inconsistent findings between sub-cohorts)
    - Blink reflex:
        -Latency RT: Normal
        -Latency LT: Normal
    - Color vision:
        -Right: Abnormal
        -Left: Abnormal
    - Visual fields:
        -Right: Abnormal
        -Left: Abnormal
    - Hearing:
        -Right: Not measured
        -Left: Not measured
    - Grip Strength:
        -Right: Unknown
        -Left: Unknown
    - Cognitive:
        -Culture Fair (non-verbal, non-arithmetical intelligence): Normal/Abnormal (inconsistent findings between sub-cohorts)
        -Digit symbol (attention and integrative capacity): Abnormal
    - Vocabulary: Abnormal
    - Recall verbal:
        -Immediate: Normal/abnormal (inconsistent findings between sub-cohorts)
        -Delayed: Abnormal
    - Perceptual motor (dexterity, coordination, discrimination, peripheral sensation):
        -Pegboard: Normal
        -Trail-making A: Normal/Abnormal (inconsistent finding between sub-cohorts)
        -Trail-making B: Normal
    - Finger-writing errors:
        -Right: Abnormal
        -Left: Normal/Abnormal (inconsistent findings between sub-cohorts)
    - Long-term memory:
        -Information: Normal
        -Picture completion: Normal
        -Similarities: Normal/Abnormal (inconsistent findings between sub-cohorts)

Subjective responses

- "Profile of mood states" score: 53 – 62.9

*Statistically significant

Author's Conclusions:

- *"Regression analysis for duration of exposure with each test in the battery and for each individual's total abnormalities did not find a single statistically significant coefficient. In contrast, the regressions of maximal estimated dose with many test scores were statistically significant, confirming the impression from comparing abnormalities in the four exposure groups. Number of abnormalities and duration of exposure appeared to be inversely related, a counter intuitive result".*
- *"....the product of dose and duration was less predictive of CNS impairment than was the magnitude of the dose."*
- *"There were 4.0 abnormalities at H_2S levels exposure below 1ppm. Exposures in the 1-5ppm range were associated with eight abnormalities. Levels between 20 and 40ppm were associated with 12 abnormalities. People exposed to above 40ppm had 18 abnormalities. The patterns of abnormality were plausible and patterns were consistent as doses increased."*
- *"Absence of effects of duration of exposure suggested that H_2S injury is all or none."*
- *"The comparison of the intensity of adverse effects, impaired CNS function, and estimated magnitude of doses suggests that the instantaneous maximal levels of H_2S determine the number of abnormalities".*

Review – Assessment:

A. Subjects:	+/- The number of subjects was deemed to be adequate for the purposes of the investigation, but was somewhat limited in terms of conventional "ecological" surveys (*i.e.*, the study more closely represented a "case-control" investigation as opposed to a full-scale epidemiological study). +/- The average age and race of subjects in the exposed cohort groups were provided, but this information was lacking for the 357 controls. - The gender of subjects was not reported. +/- Controls were *"screened to exclude previous chemical exposures and neurologic and psychiatric diseases"*. It was unclear whether the exposed groups were put under the same rigor. In addition, the nature of inclusion/exclusion criteria for these parameters was not outlined. +/- Controls were *"picked by random phone calls from voter-registration records"* and individuals in *"Group 4"* were consecutive patients; however, the manner by which individuals for Groups 2 and 3 were selected was not noted. - The location of the resident subjects comprising *"Group 3"* in relation to the source of the refinery explosion and fire was not indicated (*i.e.*, the distance and direction of the subjects' homes from the source were not provided). + Informed consent was provided. It was unclear, whether this was conducted in such a way as to prevent bias. - If withdrawals occurred, they were not listed. - The number of subjects comprising *"Group 3"* was inconsistently reported. The 'Methods' section of the report indicates 48 subjects were examined, whereas the 'Results' section suggests 44 subjects were tested. +/- Subject histories (*e.g.*, medical history, occupation, prior chemical exposures) were collected, but the manner in which the information was used was not indicated.
B. Exposure conditions:	- The precise duration of exposure was not recorded. Based on the limited information given, it would appear that exposure extended over a one-week period.

	- "Group 3" was exposed due to a *"Texaco refinery explosion and fire in October 1992"*. Information pertaining to the duration of the fire, and the relative location of the refinery with respect to residential areas from which the test subjects were recruited was lacking. - The identity and concentration of other released substances was not reported. - It was noted that *"Best estimates of exposure levels were assigned"* to exposed groups, yet the methodology supporting these estimates was not outlined. - Exposure estimates were inconsistently reported. In the 'Methods' section of the report, the concentration of H_2S was reported to range from 1-20 ppm, with a *"peak level"* of 24 ppm, whereas in the 'Results' section of the report, the range is given as 20-40 ppm. - Exposure estimates were based on area monitoring of H_2S completed both near to and distant from (*i.e.*, > 5 km) the refinery at different times during and after the fire. It was impossible to relate the area concentrations to the personal exposures the test subjects might have experienced.
C. Equipment:	NA
D. Procedural:	+/- The written questionnaire employed to obtain information pertaining to health complaints, exposure histories, *etc.* was standardized, but it was self-reporting. +/- Control subjects were selected from towns *"free of known chemical contamination"* in an effort to exclude possible confounding variables, yet information pertaining to which chemicals were considered was not reported. - *"Group 3"* subjects were studied four years post-exposure. Within this period, additional confounding variables may have been introduced.
E. Data collection:	+ Examiners were blinded with respect to subjects' exposure status. +/- Details of assessment tests were provided. The validity, sensitivity, specificity, reproducibility *etc.* of the tests were not confirmed. - Individual subject data were not provided. - Duplicate measurements were not conducted. As stated by the author *"All subjects and controls were tested once"*. - In some instances, data retrieval appeared to be either inconsistent or inaccurately reported. Within the 'Methods' section it was reported that *"Group 3"* was comprised of 48 adult subjects, but the data provided is an average of only 44 subjects. The reason for this omission is not stated. *"Group 3"* exposure levels were reported to be between 1 and 24ppm within the 'Methods' section, yet exposure levels of 20-40ppm were reported within the 'Results' section. - The characteristics of the control group (*e.g.*, sex, age, race, etc.) were lacking. - No subject data or test scores were provided for the "referent" (*i.e.*, control) group. - No numerical findings were provided. Test scores were simply recorded as "+" or "0" to signify abnormal or normal findings, respectively, relative to referent values.
F. Data analysis:	+ Statistical methods (*e.g.*, ANOVA, ANCOVA) were appropriate to limit the potential impact of confounding variables such as age, gender, height and level of education.
G. Interpretation:	- The sample cohort was not matched with controls. - For the majority of assessment endpoints, outcomes were not expressed in quantitative terms (*i.e.*, actual test scores), but rather were expressed in only qualitative terms relative to the referent group (*i.e.*, +,- or 0) or across the various cohorts.

Review - Summary:

Discussion of findings:

Exposures to H_2S evidently received during and subsequent to a refinery explosion and fire reportedly caused by a number of neurological abnormalities among nearby residents as measured by a battery of neurophysiological and neuropsychological tests. The abnormalities ranged from impairments in balance, vision and grip strength to a variety of cognitive deficits, including impaired recall, attention, and discrimination. Exposure concentrations were estimated to range from 1 to 40 ppm at the time of and subsequent to the explosion and fire.

Interpretation of the significance of the study findings should take into consideration the following:

- Exposure levels were estimated. The author states that *"best estimates of exposure levels were assigned"* to exposed groups. These exposure estimates were based on area monitoring conducted both near to and distant from (*i.e.*, >5km) the site of the refinery fire. The relative proximity of residential areas to the accident site was not stated. Consequently, it is impossible to relate the reported area concentrations to the exposure levels to which the individual subjects might have been exposed. As a result, any assessment of the dose-responsiveness of the observed neurological changes within the 'Group 3' cohort cannot be performed.
- The reported area H_2S concentrations were inconsistent within the text of the report. In the Methods section, the concentration of H_2S is stated to range from 1-20 ppm, with a peak level of 24 ppm measured in the immediate vicinity of the refinery within a few hours of the explosion and fire. In the Results section, the concentration range is listed as 20-40 ppm. No explanation for the discrepancy was provided.
- The source of the H_2S was a *"Texaco refinery explosion and fire"*. An accident of this type would release multiple chemicals. Yet, the author made no attempt to properly account for exposures to chemicals other than H_2S as possibly causing or contributing to the various neurological abnormalities reported among the test subjects. The presence of other chemicals confounds any interpretation of the findings, and renders any conclusions respecting the association between the reported neurological changes and exposure to H_2S as highly tenuous.
- For a number of parameters, test scores were inconsistent between the two sub-cohorts comprising Group 3 (*i.e.*, balance with eyes closed, Culture Fair performance, immediate verbal recall, Trail-making A, finger writing errors, and long-term memory for similarities). Thus, any classification of the scores as "normal" or "abnormal" is questionable Yet, the author reports the scores for most of these parameters to be "abnormal", without apparent basis and with no explanation for the discrepancies.
- No individual subject data were provided and no numerical findings were supplied. Findings were simply denoted as "+" or "0" on the basis of group mean scores to signify "abnormal" or "normal" responses, respectively, relative to the referent group. It was not possible to determine the inter-subject variability in test scores for any parameter. Given that any exposure to H_2S as a result of the refinery explosion and fire likely varied over at least two orders of magnitude, the provision of individual subject data (or at the very least, an indication of the variance surrounding the test scores) would have assisted in the interpretation of the findings, particularly in terms of assessing the strength of the exposure-response association.
- An excessive lag period occurred between the time of the refinery explosion and fire and the testing of subjects, with more than 3 years elapsing. This confounds any interpretation of the results since other events and/or exposures occurring subsequent to the refinery explosion and fire could have contributed to the reported neurological changes. In addition, the long lag period undermines confidence in the results for outcomes (*i.e.*, health complaints) that were based on self-reported questionnaires requiring recall of events that occurred more than 3 years previously.

Confidence index:

The level of confidence that could be assigned to the study findings and conclusions was deemed to be low. Several weaknesses in experimental design, conduct and reporting were evident. The weaknesses included.

- Lack of reliable exposure estimates. Exposure to H_2S was estimated on the basis of measurements taken near and distant from the source of emissions at different times following the refinery explosion and fire, but information on the location of the subjects' homes relative to the source and to the sites of measurement was not provided. Accordingly, the exposure of the individual subjects is unknown.
- Excessive lag time between the refinery fire and explosion and the testing of the subjects. The lapse of more than 3 years between the events seriously

confounds the interpretation of the findings.

- Failure to report numerical findings. Results were simply expressed as "+" or "0" to signify abnormal or normal scores relative to control values. This precluded critical interpretation of the findings.
- Failure to account for chemical exposures apart from H_2S that may have resulted from the refinery fire and explosion and might have contributed to the observed neurological outcomes.
- Inconsistent and possibly biased reporting of findings. For several parameters, changes were inconsistent between the two sub-cohorts comprising the test group, yet results were commonly listed as "abnormal" without explanation of the discrepancies.

Review – Confidence index ranking:

Low

Document Review

Author:	Kosmider, S; Rogala, E; Pacholek, A	**Internal ID:**	223
Title:	Electrocardiographic and histochemical studies of the heart muscle in acute experimental hydrogen sulfide poisoning		
Year:	1967		
Abstract:	*"In experiments with rabbits, electrocardiographic changes in the course of hydrogen sulfide poisoning have been studied. Treatment of arrhythmia induces with hydrogen sulfide was attempted, and the reactions for some of the enzymes in the heart muscle were studied. Acute hydrogen sulfide poisoning causes disorders in the ECG tracings, especially pertaining to repolarization of the ventricles. In subacute poisoning extrasystoles of various origin predominated. Arrhythmia in the course of hydrogen sulfide poisoning was abolished by intravenous injections of compounds binding calcium ions, namely sodium citrate. Hydrogen sulfide poisoning causes a depression of ATPase activity in the myocardial membranes, which may affect active transport, especially of Na and K, from the blood to the sarcoplasm. Activity of the respiratory enzymes is also depressed, undoubtedly affecting cellular respiration."*		
Objective:	To investigate the effects of acute and subacute inhalation of H_2S on heart muscle activity in rabbits by means of electrocardiographic and histochemical analyses.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.1 mg/L = 71ppm (whole body)	(a) Single/ 1.5hr (b) Repeated/ 0.5hr per day for five days (c) Control	Rabbit	Not specified (Stated to be "mixed breed" only)	Adult (I yr)	Both	37. Divided into 3 groups: (a) 10 (b) 17 (c) 10	Not specified	Not specified	71ppm

Observations:

- Acute exposure:
    - The animals reportedly lost consciousness
    - Electrocardiogram (ECG) abnormalities:
        - Heart rate (HR): 3/10 slowed; 4/10 accelerated
        - P-wave: No change
        - PQ interval: No change
        - QT interval: No change
        - Change in QRS complex: 1/10 abnormal
        - ST segment: No change
        - T wave: 9/10 abnormal
        - Pathologic angle of electric axis: 1/10 abnormal

        [The severity of the changes was not reported]

- Subacute exposure:
    - No clinical signs reported
    - Arrhythmias (in the form of ventricular extrasystoles and bigeminal rhythm): Present and persisted for several days post-exposure.
    - ECG abnormalities:
        - HR: 4/17 slowed; 1/17 accelerated

- P-wave: 2/17
- PQ interval: No change
- QT interval: No change
- Change in QRS complex: 15/17 abnormal
- ST segment: No change
- T wave: 15/17 abnormal
- Pathologic angle of electric axis: 7/17 abnormal

[The severity of the changes was not reported]

- Histochemical analyses:
  - ATP phosphohydrolase activity: Decrease in blood vessels and sarcolemma of muscle fibers. No change in sarcoplasm.
  - $NADPH_2$ oxidoreductase activity: Decrease in sarcoplasm and vascular endothelium.

*Statistically significant

Author's Conclusions:

- *"The character of electrocardiographic abnormalities in hydrogen sulfide poisoning depends on duration of exposure and degree of poisoning."*
- *"Acute poisoning causes changes in ventricular repolarization, and subacute poisoning causes disorders of cardiac rhythm."*
- *"Histochemical studies showed a decrease in the intensity of the reactions for ATP phosphohydrolase and $NADPH_2$ oxidoreductase in the myocardial cells, indicating a direct toxic effect of hydrogen sulfide on the cells."*
- *"Our observations indicate that secondary changes in the concentration of biologically active intracellular calcium induced by hydrogen sulphide may be the cause of the [cardiac] disorders".*
- *"The histochemical studies showed that hydrogen sulfide acts directly on the myocardial cells, not, as supposed until now, through the vegetative nervous system".*
- *"The mechanism of the disorders of cardiac rhythm in hydrogen sulphide poisoning is not clarified. Our observations indicate that hydrogen sulphide poisoning produces mineral disorders in the body, although this problem requires further investigation".*

Review – Assessment:

A. Test animals:	- The experiment was conducted on rabbits of mixed breed and sex. The distribution of sexes across exposure groups was undefined. + Number of test animals was judged to be adequate for the purposes of the study. + Exposed animals were adults. - The breeding facility from which the animals were obtained was not noted. - Pre-test health status of animals was not indicated. + The 20% weight variation of test animals was within recommended guidelines.
B. Exposure conditions:	- The source of the H_2S gas was not specified. - Only nominal exposure concentrations were recorded. There is no indication that the target exposure concentrations were analytically confirmed. - No justification was provided for dose selection. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could

	potentially alter the duration of exposure. + The exposure period was precisely defined. + The mode of administration was whole body. - Exposure concentrations, airflow, temperature and humidity within the exposure chamber were not monitored.
C. Housing/Feeding:	- No information pertaining to housing conditions was provided (*i.e.*, temperature, humidity and photoperiod). - No information respecting animal caging (*i.e.*, type, dimensions, bedding material) was provided. +/- Test animals were fed a "*standard diet*" before and during the experiment. - No details concerning feeding restrictions during exposure were given. - No details concerning water supply were provided.
D. Equipment:	- Details concerning the exposure chamber (*e.g.*, type and dimensions) and gas delivery system were completely lacking.
E. Procedural:	- No acclimation period or pre-test conditions (*i.e.* quarantine, disease treatment, etc.) were specified. - It was unclear whether test animals were randomly assigned to exposure groups. - Control electrocardiograms for an undefined number of animals were conducted 10 days before exposure. It was not apparent if the handling of the control animals mimicked that of the exposed animals. - There is no indication that control exposures (*i.e.*, 0 ppm) were performed. Control animals were evidently used for the histochemical analyses only. - Controls were not matched with respect to gender. - A single exposure concentration only was tested (*i.e.*, 71 ppm). Testing guidelines generally recommend use of graded exposure levels to permit assessment of dose-responsiveness. - Enzyme activities were determined on the basis of histochemical analyses, and not through conventional enzyme assay methodologies. - Conventional measures of toxicity (*e.g.*, body weights, clinical observations, gross pathology, organ weights) were largely ignored. +/- The study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	- Observations in the acute study were made 1.5hrs after the animals had lost consciousness. The study might have yielded additional behavioral information had symptoms been monitored continuously. +/- Individual animal data respecting electrocardiogram abnormalities were provided; individual data for all remaining endpoints monitored were not available. - Sample ECG tracings were provided; however, the exhibits were not adequately labeled (*i.e.*, animal identification was not indicated), and were of limited resolution. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
G. Data analysis:	- No statistical analyses were conducted.
H. Interpretations:	- Bias might have been introduced due to several deficits in the experimental design (*e.g.* absence of control exposures, lack of randomization, etc.). - The severity of the cardiac abnormalities was not indicated. This renders interpretation of the findings difficult, especially since the abnormalities observed are also likely to occur spontaneously in rabbits. The absence of a control exposure group simply adds to the difficulty in interpretation.

Review - Summary:

Discussion of findings:

Acute and subacute exposure of rabbits to H_2S reportedly caused electrocardiographic and histochemical changes in the heart muscle, as evidenced by changes in ECG tracings and decreased enzyme activities. Rabbits exposed to 71ppm H_2S for 1.5 hours showed changes in ventricular repolarization. Subacute exposure of rabbits to 71 ppm H_2S for 30 minutes per day over a 5-day period induced persistent arrthymias, as evidenced largely by the appearance of ventricular extrasystoles. Histochemical analyses of heart muscle obtained post-exposure revealed changes in the activities of ATP phosphohydrolase and $NADPH_2$ oxidoreductase. Clinical signs of intoxication followed acute exposure, with the animals losing consciousness. No clinical signs were reported for the rabbits as a result of subacute exposure.

Interpretation of the significance of the study findings was limited by deficiencies in study design, conduct and reporting that undermined the scientific integrity of the investigation (see below). Apart from the low level of confidence that can be assigned to the findings, interpretation should take into consideration the following:

- The dose-responsiveness of the observed changes in cardiac rhythm and heart muscle enzyme activities could not be determined owing to the use of a single exposure concentration only. Confidence in the study findings would have been improved had additional exposure groups been included, provided a consistent dose-related pattern presented for each response.
- The changes in heart muscle enzyme activities were determined on the basis of histochemical analyses of tissue sections, involving differential staining techniques. The enzyme activities were not determined using conventional assay methodologies, which are more specific, more sensitive, and more reproducible. The incidence and severity of the changes in enzyme activities could not be assessed on the basis of the limited information provided in the report.
- Detailed interpretation of the ECG tracings and abnormalities was not attempted. However, limitations in the amount of information supplied (*i.e.*, lack of control data, lack of adequate identification of tracings, limited numbers of tracings, limited resolution of tracings) would likely preclude critical analysis. Accordingly, verification of the study findings and conclusions could prove to be difficult.
- The authors admit that the mechanism(s) underlying the reported changes in cardiac rhythm are *"not clarified"* and *"require further investigation"*, suggesting that the study findings should be considered preliminary in nature.
- Clinical observations were limited, with the major focus of the study aimed at understanding of the mechanism of action of H_2S at the sub-clinical (*i.e.*, tissue and cellular) level. However, the findings are of some interest and relevance from a clinical perspective since the heart might be expected to be a target tissue for H_2S toxicity given its high oxygen demand and its reliance on normal oxidative metabolism and energy production.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to seriously undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Complete lack of detail concerning the exposure chamber and gas delivery system.
- General lack of detail concerning exposure conditions.
- Lack of evidence to suggest that stated exposure concentrations were actually achieved (*i.e.*, no analytical confirmation of exposure concentrations).
- Complete lack of detail concerning animal housing and husbandry.
- General lack of control data, especially with respect to electrocardiographic findings.
- General lack of data respecting enzyme activities (*i.e.*, reported changes in ATP phospohydrolase and $NADPH_2$ oxidoreductase activities could not be verified, nor could the severity of the changes be determined).
- Lack of statistical analyses of the study findings.

Review – Confidence index ranking:

Low

Document Review

Author:	Lopez, A; Prior, M; Yong, S; Albassam, M; Lillie, L	**Internal ID:**	231
Title:	Biochemical and cytologic alterations in the respiratory tract of rats exposed for 4 hours to hydrogen sulfide		
Year:	1987		
Abstract:	*"Fischer-344 rats were killed by exsanguination 1, 20, and 44hr after a single 4-hr exposure to an atmosphere of 0,10, 200, and 400ppm of hydrogen sulfide (H_2S). Alterations in the activities of lactate dehydrogenase and alkaline phosphatase, and cytomorphology of epithelial cells in fluids obtained by nasal and broncoalveolar lavage were used as indicators of cell injury. Changes in the number of leukocytes were used as indicators of inflammatory response, and changes in the concentration of protein were used as indicators of altered vascular permeability. Inhalation of H_2S resulted in 139, 483, and 817% increased cellularity in the nasal cavity of rats exposed to 10, 200, and 400ppm, respectively. This was due to marked exfoliation of degenerated epithelial cells and exudation of neutrophils. The high dose of H_2S resulted in a moderate increase in lactate dehydrogenase and protein in nasal passages; values returned to baseline levels 20hr later. Bronchoalveolar cell counts were decreased in rats exposed to 400ppm and unchanged in those exposed to 10 and 200ppm. Enzymatic activities in lung lavage fluid were moderately elevated (up to 90%), yet protein concentrations were increased by more that 3000% and remained significantly elevated up to 44hr after exposure to 400ppm. It was concluded that inhalation of H_2S has a severe cytotoxic effect on the nasal epithelium and a severe edematogenic effect on lung parenchyma. These results are in agreement with autopsy findings of individuals killed by accidental exposure to H_2S-containing sour gas."*		
Objective:	To evaluate the early injury and inflammatory response occurring in the respiratory tract of rats after a single 4-hr exposure to 0, 10, 200, or 400ppm of H_2S. A series of cellular and enzymatic markers obtained through nasal and bronchoalveolar lavage were monitored at 1, 20 and 44 hours post-exposure.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0, **10**, 200 or 400 ppm (whole body)	Single/ 4hr exposure	Rat	Fischer-344	10-week	Male	60. 12 rats per exposure group. (4 rats per group sacrificed at each of three post-exposure intervals).	Not specified	Not specified	10ppm (?)

Observations:

- Biochemical analysis of naval lavage fluid:
  - Protein concentration: Transient 800% increase at 400ppm H_2S. Change was apparent at one hour post-exposure only … protein concentration had returned to normal by 20 hours post-exposure. No effect at **10** or 200 ppm.
  - Lactate dehydrogenase activity: Transient 320% increase at 400ppm H_2S.* No change at **10** or 200 ppm.
  - Alkaline phosphatase activity: No change.

- Biochemical analysis of bronchoalveolar lavage fluid (BAL):
  - BAL Protein concentration:
    -400 ppm: Transient 3000% increase*, however, by 44 hours post-exposure increase was only 369%.*
    -**10** and 200 ppm: No change.
  - BAL Lactate dehydrogenase (LDH) activity:
    -400 ppm: Transient 89% increase*, however, by 44 hours post-exposure increase was only 22%.*
    - No change at **10** or 200 ppm.

- BAL Alkaline phosphatase activity:
  - -200ppm: Transient 6% increase, however, by 20hours post-exposure the level was 35% below baseline*, and by 44 hours this was further reduced to 63% of the baseline value.*
  - -400 ppm: Transient 47% increase*, however, by 20 hours post-exposure levels were lower than baseline values.*
  - -**10** ppm: No change
- BAL γ-glutamyl transpeptidase activity:
  - -400 ppm: 933% increase*
  - -**10** and 200 ppm: No change

- Serum biochemical analysis:
  - Serum protein: No change.
  - Serum LDH: No change.
  - Serum alkaline phosphatase activity:
    - -400ppm: 15% decrease at 20 hours post-exposure; 17% decrease at 44 hours post-exposure.

- Nasal lavage fluid cellular composition:
  - Total cell concentration:
    - -**10**ppm: Transient 139% increase, within 20 hours post-exposure concentration returned to baseline values*
    - -200ppm: Transient 483% increase, within 20 hours post-exposure concentration returned to baseline values*
    - -400ppm: Increased by 817% one hour post-exposure, 501% 20 hours post-exposure, 73% 44 hours post-exposure.*
  - Initial elevated cellularity due to increase in desquamation of nasal epithelium. At 20 hours post-exposure exudation of polymorphonuclear leukocytes was primary cell type.
  - Note that the changes in nasal lavage cellularity could not be verified due to reporting inadequacies (*i.e.*, changes were reported in terms of descriptive text only).

- Nasal cytopathology:
  - Cytoplasmic vacuolation, pyknosis, karyorrhexis, cytoplasmic constriction between basal region and terminal plate, and finally separation of ciliated tufts. Exposure-response characteristics not specified.

- Brocholalveolar lavage fluid cellular composition:
  - Number of nucleated cells:
    - -**10**ppm: No change
    - -200ppm: No change
    - -400ppm: 42% reduction at PEH 1*; No change at PEH 20; 33% increase at PEH 44

- Pulmonary cytopathology:
  - No features of cell degeneration or ciliocytophthoria

- Clinical responses:
  - 400ppm: Moderate lethargy, rapid recovery
  - No notable effect on respiratory movements.
  - No deaths

- No clinical signs at **10** or 200 ppm.

*Statistically significant

Author's Conclusions:

- *"..inhalation of high doses of H_2S induce severe but transient nasal injury and pulmonary edema"*
- *"..poor dose-effect relationship..once a threshold is reached, lesions appear rapidly in a dramatic fashion."*
- *"The early cytotoxic changes...were similar to those...of other sensory irritant gases....vacuolization, ciliocytophthoria, and sloughing of nasal cells"*
- *"The cell count of nasal lavage was the only parameter significantly affected by inhalation of a low concentration (**10ppm**) of H_2S."*
- *"Increased activity of LDH in nasal lavage fluid following 400ppm H_2S could have resulted from injured epithelial cells or from exudated plasma."*
- *"Increased activities of LDH and ALP in BAL fluid indicated that, in addition to the nasal damage, inhalation of H_2S gas also produces cytotoxic changes in the pulmonary epithelium. However, the magnitude of enzymatic and cytologic changes in BAL after 400ppm of H_2S was considerably milder than for other pneumotoxicants."*
- *"...findings suggest that H_2S is considerably less cytotoxic for bronchoalveolar epithelial cells than it is for nasal epithelium. However, concentrations of H_2S reaching the bronchoalveolar region should be expected to be lower than those in the nasal cavity."*
- *"The origin and significance of altered γ-GT in toxic lung injury requires further study."*
- *"..extraordinary increase in protein concentration in BAL fluid following 400ppm of H_2S was a good indicator of the edematogenic properties of this gas....Elevated protein concentrations in the bronchoalveolar space up to 44 hr after exposure suggest that either protein continues to exude for some time after initial injury or that absorptive and/or other removal mechanisms in the lung may be altered."*
- *"Whether H_2S-induced pulmonary edema is inflammatory, cardiogenic, or neurogenic , or results from a combination of different pathogenetic mechanisms remains to be elucidated."*
- *"Although a single exposure of up to 400ppm for 4 hr appeared to have only transient effects on the integrity of the respiratory membrane, a long-term functional abnormality cannot be ruled out as possible secondary sequelae of the edematogenic and erosive effects of H_2S inhalation...altered bacterial clearance...may result in an increase susceptibility to infections."*
- *"It was concluded that inhalation of H_2S has a severe cytotoxic effect on the nasal epithelium and a severe edematogenic effect on lung parenchyma".*

Review – Assessment:

A. Test animals:	- The number of test animals was somewhat limited, with results based on 4 rats per exposure group at each of three different sacrifice times. Guidelines for acute toxicity testing generally recommend use of 10 rats per exposure concentration. +/- The principle characteristics of test animals (*e.g.*, specie, strain, age, and sex) were adequately reported, however, information pertaining to weight variation and pre-test health status would have been beneficial. - Only male rats were exposed + The source of the test animals was noted. - Rats are obligate nasal breathers, hence, nasal injury may be observed at lower H_2S levels than in other species.
B. Exposure conditions:	+/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure. +/- Since the exposure system could only expose three groups in a single trial, two separate trials were performed within two days, each with a separate control group. + The exposure device was thoroughly described. + The actual gas concentrations were determined and recorded. Gas concentrations were monitored 3 times an hour and

	analyzed by gas chromatography + The exposure chamber was maintained at negative pressure in compliance guideline recommendations.
C. Housing/Feeding:	+/- The material from which cages were constructed was noted (*i.e.*, stainless steel, wire mesh bottomed). However, the dimensions of these cages were not included in the description. + Test animals were individually housed, therefore, clear observation of each animal was possible. + Commercial pelleted rat feed and water were fed *ad litum* to test animals in accordance with OECD guidelines. - Feeding restrictions imposed during exposure were not noted. + Animals were housed in an environmentally-controlled room (*e.g.* 19 to 24 °C; 30 to 70% humidity; monitored photoperiod) + Air flow was recorded and complied with OECD guidelines (*i.e.*, 12 to 15 changes per hour)
D. Equipment:	+ All exposure, monitoring and analysis equipment used was adequately described.
E. Procedural:	+ Animals were acclimatized for two weeks prior to exposure. + Acclimatization to the noise and confinement within the exposure chamber was conducted for 4hr during 3 consecutive days prior to exposure. + Rats were randomly allotted to exposure groups and to cage location within the gas chamber. The method of randomization (*e.g.*, table of random numbers, computer generated) was not noted. + Controls were also placed within chambers to account for physiological responses associated with this stress. - Unclear whether technicians and handlers were blinded to exposure conditions. - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- No pre-determined scales were used to assess clinical responses. - Raw data for certain parameters (*e.g.*, serum protein levels) were not reported.
G. Data analysis:	- Individual data were not provided for each test animal, thereby limiting independent assessment of the findings. + A one-way analysis of variance and Student's *t* test was used to determine statistical significance of findings.
H. Interpretation:	- Changes were confined largely to animals exposed to 400 ppm. Accordingly, the conclusion that "*inhalation of H_2S has a severe cytotoxic effect on the nasal epithelium and a severe edematogenic effect on lung parenchyma*" must be interpreted with caution. - Effects appeared to be localized to the respiratory tree. Serum biochemical markers were largely unchanged by exposure. - Expected response for most of the enzymatic markers would be an increase in activity following exposure. However, for certain enzymes at certain times post-exposure (*e.g.*, serum ALP, BAL ALP at 20 and 44 hours post-exposure), activities were decreased relative to controls, with no explanation.

Review - Summary:

Discussion of findings:

An acute exposure to a high concentration of H_2S (*i.e.*, 400 ppm) clearly affected the integrity of the respiratory membrane, as evidenced by changes in cellular and enzymatic markers of cytotoxic and edematogenic responses. At 400ppm, H_2S induced transient responses in most of the parameters monitored, reflecting the irritant properties of the gas. Recovery was seemingly rapid.

Acute exposure to 200ppm H_2S, induced a transient 6% increase in brochoalveolar alkaline phosphatase (ALP) activity, possibly indicative of cytotoxic damage. However, recovery was rapid, and ALP activity rebounded considerably (*i.e.*, within 44 hours, activity of the enzyme was 63% below the baseline value). The only other response reported at 200 ppm was a transient increase in the cellularity of nasal lavage fluid. No other indications of cellular damage were found.

The only significant change noted in response to10ppm H_2S exposure for 4 hours, was a transient increase in the absolute number of epithelial cells detected in the nasal lavage fluid of exposed rats. The change was apparent only at one-hour post-exposure. Details of the change were lacking since the response was presented as part of the descriptive text only.

Interpretation of the significance and relevance of the study findings to the human condition should take into consideration the following:

- The injury to the respiratory tract identified through the various biochemical and cytological markers was dose-related. Notable responses were generally confined to the animals exposed to 400 ppm of H_2S. The responses were consistent with the known irritant properties of the gas, and presented as cytotoxic effects on the nasal passages and edematogenic effects within the lung.
- At 10 ppm of H_2S, the only parameter significantly affected was the nasal lavage cell count. The response was transient. The authors cautioned that the toxicological significance of the response should not be overestimated *"since dramatic changes can rapidly be restored due to the remarkable reparative capacity of respiratory epithelium"*. All other parameters remained unchanged at 10 ppm.
- Apart from transient lethargy at 400 ppm, clinical signs of toxicity were not evident either during or following exposure. No indication of respiratory distress was apparent even among the rats exposed to 400 ppm for four hours.
- Rats are obligate nasal breathers. Accordingly, the cytotoxic effects on the nasal passages that followed exposure may over-represent the responses that might be witnessed in humans under similar exposure conditions. However, the edematogenic effects on the lung parenchyma may be particularly relevant to humans since they are consistent with the pulmonary edema frequently observed among persons severely poisoned or killed as a result of accidental exposure to H_2S.
- The focus of these study was to document the histological changes in the respiratory tract that result from H_2S exposure. This includes outlining the differential sensitivity of cells lining the respiratory pathway and detailing the reparative capacity of affected cells. Clinical symptoms may only be immediately apparent following exposure to higher H_2S levels; however, histological changes which may increase the susceptibility of the airways to infections or further damage are important toxicological changes. Further study is needed to better understand the reparative processes.

Confidence index:

The confidence index of the study was judged to be moderate. The study design, conduct and reporting were judged to be adequate for the stated purposes of the investigation. Added confidence in the findings and conclusions might have been achieved through:

- Use of larger group sizes of test animals. (The use of only 4 rats per exposure concentration at each sacrifice time was judged to be somewhat restrictive).
- Use of both sexes of test animals. (The study was performed using male rats only. Guidelines generally recommend use of both sexes).

Review – Confidence index ranking:

Moderate

Document Review

Author:	Lopez, A; Prior, M; Lillie, LE; Gulayets, C; Atwal, OS	**Internal ID:**	232
Title:	Histologic and ultrastructural alterations in lungs of rats exposed to sub-lethal concentrations of hydrogen sulfide		
Year:	1988		
Abstract:	*"Fischer-344 rats were killed 1, 18, and 42 hr after a single 4-hr exposure to an atmosphere of 0, 116, or 615 mg m^{-3} of hydrogen sulfide (H_2S). Lungs, fixed by the intratracheal route, were examined by light and electron microscopy. Histologic changes were transient and mainly present in rats exposed to 615 mg m^{-3} H_2S. Lesions included severe but transitory pulmonary edema and fibrinocellular alveolitis which was restricted to the proximal alveolar region of the lung. Electron microscopically, ciliated bronchiolar cells were the only cells that developed necrosis; they were rapidly replaced by mitosis. Alveolar endothelium had cytoplasmic blebs, but alveolar epithelium had minor changes. No mast cell degranulation was detected in lungs with edema. A 4-hr exposure to 615 mg m^{-3} is markedly edematogenic for the lung but only moderately cytotoxic for pulmonary cells."*		
Objective:	To study histological and ultrastructural alterations in the lungs of rats exposed to a single edematogenic concentration of H_2S.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; **83** ppm; 439 ppm	Single exposure / 4 hours. Rats were subsequently sacrificed at either 1, 18 or 42 hours post-exposure (designated PEH 1, 18 and 42, respectively)..	Rat	Fischer-344	10-week	Male	36. 12 per exposure group, with 4 rats per group killed at each sampling time.	Not specified.	NA	**83** ppm

Observations:

- Histological:
  - **83** ppm:
    - Transient, perivascular edema. (Minor)
  - 439 ppm:
    - Perivascular edema. (Severe)
    - Distended pulmonary lymphatics filled with proteinaceous material (Severe)
    - Changes in airways and alveoli
      - PEH 1: In the bronchioles and proximal alveolar region, polymorphonuclear leukocytes (PMN), proteinaceous fluid, fibrin strands, and a few epithelial cells were noted.
      - PEH 18:
        - Disappearance of perivascular fluid.
        - Elevated number of mitotic figures in the bronchiolar epithelium
        - Continued infiltration of alveolar interstitium and bronchiolar submucosa by PMN.
        - Moderate exudative alveolitis.
      - PEH 42:
        - Cessation of PMN infiltration of bronchiolar submucosa
        - Increased number of enlarged pulmonary alveolar macrophage.

- Hyperplasia of alveolar type II cells

- Ultrastructure:
  - **83** ppm: Mild transient.distension of the perivascular space.
  - 439ppm:
    - Transient distention of the axial and alveolar interstitium by edematous fluid.
    - Transient distention of perivascular space.
    - Vesiculation of endothelial cells, hyperplasia of smooth cell organelles
    - Low electron-dense patches of perivascular edema.
    - Leukocyte infiltration.
    - Transient subendothelial blebbing.
    - Topographic distribution of injured cells within bronchioles was random.
    - Depletion of lamellar bodies in alveolar type II cells.
- Clinical observations: None recorded

*Statistically significant

Author's Conclusions:

- *"Inhalation of 615mg m^{-3} /4-hr H_2S has only a transient effect on the lungs of rats."*
- *"Histologic changes were transient and mainly present in rats exposed to 615mg m^{-3} H_2S. Lesions included severe but transitory pulmonary edema and fibrinocellular alveolitis which was restricted to the proximal alveolar region of the lung."*
- *"Ciliated bronchiolar cells are the main target cell for acute H_2S toxicity in the lung; however, necrotic cells are rapidly replaced by mitosis"*
- *"Alveolar endothelium had cytoplasmic blebs, but alveolar epithelium had minor changes."*
- *"No mast cell degranulation was detected in lungs with edema."*
- *"...the overall type of injury and host response to H_2S differs from that produced by other gases, in that no injury occurs in the air-blood barrier"*
- *"A 4-hr exposure to 615mg m^{-3} is markedly edematogenic for the lung but only moderately cytotoxic for pulmonary cells."*

Review – Assessment:

A. Test animals:	- Number of animals was limited (*i.e.*, 4 rats per exposure concentration per sacrifice time) in relation to guideline recommendations. +/- The principle characteristics of test animals (*e.g.*, specie, strain, source, age, weight variation and sex) were reported. - Pre-health status of animals was not reported. + Weight variation of the animals at study initiation (*i.e.*, 4%) was well below the maximum set forth by testing guidelines. + The source of the test animals was noted. - Only male rats were exposed.
B. Exposure conditions:	+/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure. + The exposure device was adequately described, with more complete details reportedly available in companion papers (Lopez

	et al., 1987). Gas flow rates and chamber pressures were stated. + Target gas concentrations were monitored and analytically confirmed. Gas concentrations were monitored 4 times an hour and analyzed by gas chromatography. + The exposure chamber was maintained at negative pressure in accordance with guideline recommendations.
C. Housing/Feeding:	+ The material from which cages were constructed was noted (*i.e.*, stainless steel, wire meshed bottomed), but the dimensions of these cases were not included in the description. + Test animals were individually housed, therefore, clear observation of each animal was possible. + Commercial pelleted rat feed and water were fed *ad libitum* to test animals in accordance with guideline recommendations. +/- Source of feed was given, but source of water supply was not provided. - Feeding restrictions imposed during exposure were not noted. + Animals were housed in an environmentally-controlled room (*i.e.*, 22+/- 2°C; 50 +/- 20% humidity; monitored photoperiod) + Animals were maintained in accordance with guidelines provide by the Canadian Council of Animal Care.
D. Equipment:	+ All exposure, monitoring and analysis equipment used was adequately described.
E. Procedural:	+ Animals were acclimatized for ten days prior to exposure. - Pre-test health including quarantine, and disease treatment were not discussed. +/- Rats were randomly allocated to exposure groups, but the method of randomization (*e.g.*, table of random numbers, computer generated) was not noted. - Whether rats were randomly assigned to cage position within gas chamber was not reported. + Controls were also placed within chambers to account for physiological responses associated with stress. - Unclear whether technicians and handlers were blinded to exposure conditions. + Conventional measures of toxicity, including clinical signs, body weights and gross pathology were not performed. - Only sections of left lung were taken for histopathological and electron microscopic analyses. - No indication the study was performed under Good laboratory Practice (GLP) conditions.
F. Data collection:	- No clinical responses were recorded. - Individual raw data were not provided for each test animal, thereby, limiting the independent assessment of findings. - The number of animals which histological or ultrastructural changes were observed within was not noted. + Photomicrographs of histological slides were provided to illustrate findings.
G. Data analysis:	- No statistical analyses were conducted.
H. Interpretations:	+ The reversibility of observed changes was explored by conducting three separate sacrifices (1, 18, and 42-hours post exposure).

Review - Summary:

Discussion of findings:

Histological and ultrastructural changes in the lungs of rats following a 4-hour exposure to **83** ppm or 439 ppm of H_2S were examined using light and electron microscopy. A primary aim of the study was to provide better understanding of the etiology and progression of the pulmonary edema which can follow H_2S exposure.

Changes to the morphological architecture of the lungs were dependent on exposure concentration and the time elapsed post-exposure. At **83** ppm, changes were relatively minor, and consisted primarily of perivascular edema involving the large- and medium-sized pulmonary vessels noted on light microscopic examination, and mild distension of the perivascular space noted at the ultrastructural level. The changes resolved quickly following cessation of exposure. No other changes to the lungs following exposure to **83** ppm were reported. Exposure to 439 ppm resulted in various histological and ultrastructural changes, with the ciliated bronchiolar cells being the main target cells. Marked perivascular edema of the pulmonary vessels also was observed. Again, changes were transient, with most affected tissues/cells determined to be in a reparative state within 48 hours post-exposure, as demonstrated by increased numbers of mitotic figures. The findings revealed a specific topographical distribution of lesions in the lung, with selective injury to the terminal bronchiolar and proximal alveolar regions predominating.

Interpretation of the significance of the findings should take into consideration the following:

- The observed changes were dose-dependent. Only minor lesions were detected following exposure to **83** ppm at both the histological and ultrastructural levels.
- The observed changes were generally transient, with recovery evident within 48 hours post-exposure.
- Presumably, no obvious clinical signs of respiratory distress accompanied the histological and ultrastructural changes. No clinical signs of intoxication were recorded.
- Confidence in the findings is limited somewhat by the use of restricted numbers of animals, with lung tissue obtained from only 3 rats per exposure concentration at each sampling time. In addition, the incidence of the lesions could not be determined on the basis of the information supplied (*i.e.*, the number of rats afflicted with any given lesion was not stated).
- Rats are obligate nose breathers. The nature and severity of the histological and ultrastructural changes might differ among species, such as humans, with different breathing patterns.

Confidence index:

The confidence index of the study was judged to be moderate. Study design, conduct and reporting were judged to be adequate for the purposes of the study. Confidence in the findings could have been improved by:

- Use of larger numbers of test animals.
- Use of both sexes of rats.
- Presentation of a numerical summary showing incidence rates by treatment group for each of the major lesions observed.
- Addition of routine measures of toxicity, notably clinical observations, to permit better assessment of the clinical significance of the microscopic changes.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Lopez, A; Prior, M; Yong, S; Lillie, L	**Internal ID:**	233
Title:	Nasal lesions in rats exposed to hydrogen sulfide for four hours		
Year:	1988		
Abstract:	*"Fischer-344 rats were exposed for 4 hours to 0, 14, 280, or 560 mg of hydrogen sulfide m^{-3} and killed 1, 18, or 44 hours later. We evaluated the nasal epithelial cells and determined the anatomic distribution of lesions. Inhalation of 560 mg of hydrogen sulfide m^{-3} induced necrosis and exfoliation of respiratory and olfactory mucosal cells, but not squamous epithelial cells. The anatomic distribution of lesions was midway along the nasal passages involving nasal and maxillary conchae, but not ethmoidal conchae. Injured respiratory mucosa repaired rapidly, whereas olfactory mucosa continued to exfoliate at 44 hours after exposure."*		
Objective:	To determine the types of cells and the anatomic areas of the nasal cavity that are injured in rats after a single 4hr exposure to H_2S at levels of 0,10, 200, or 400ppm. (Note that additional details respecting the design and execution of the study can be found in Lopez *et al.* (1987 – Study ID 231).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0, **10**, 200 or 400 ppm (whole body)	Single/ 4 hours	Rat	Fischer-344	10-weeks	Male	60. 12 rats per dose group	Healthy (specific-pathogen free)	200ppm	400ppm

Observations:

- Occurrence of nasal lesions:
  - 0 ppm, **10** ppm; 200 ppm: A few rats exhibited sporadic "background" nasal lesions.
  - 400 ppm: Rats exhibited comparable and repeated lesions. (26/48)
  - Minimal dose-response relationship.

- Distribution of lesions in nasal cavity
  - Lesions were multifocal and restricted to specific areas of the nasal cavity
  - Sector 1/Rostral sector: Not affected
  - Sector 2 and 3/Middle sectors: Most severely and repeatedly affected
  - Sector 4/Caudal sector: Less affected.

- Response by cell type:
  - Squamous epithelium: No change
  - Respiratory epithelium: Necrosis and exfoliation peaked at 1 hour post-exposure. 44hrs post-exposure respiratory epithelium was in a reparative stage
  - Olfactory epithelium: Necrosis and disruption of cellular architecture at 1 hour post-exposure. At 44 hr post-exposure, cells continued to degenerate.

- Clinical signs:
  - 400 ppm: Lethargy and epiphora

- No clinical signs at **10** or 200 ppm

*Statistically significant

Author's Conclusions:

- *"..in a 4-hour exposure, concentrations of >200 ppm of H_2S^3 are needed to induce detectable histological lesions in the nasal cavity."*
- *".. only rats exposed to 400 ppm had comparable and repeated lesions in the nasal cavity"*
- *"A few rats exposed to 10 (n=4) or 200 (n=2) ppm and 1 rat that was not exposed to H_2S had sporadic background lesions".*
- *"The anatomic distribution of these lesions corresponded with that previously described in rats and mice exposed to other irritant gases"*
- *"Lesions…developed in the middle area of the nasal passages (sectors 2 and 3), whereas most rostral and caudal aspects of the nasal passages (sectors 1 and 4) were mildly affected or unaffected. The reason why sectors 2 and 3 were most vulnerable to H_2S or to other toxic gases requires further investigation."*
- *"Inhalation of 400 ppm of hydrogen sulfide induced necrosis and exfoliation of respiratory and olfactory mucosal cells, but not squamous epithelial cells."*
- *"Injured respiratory mucosa repaired rapidly whereas olfactory mucosa continued to exfoliate at 44 hours after exposure."*

Review – Assessment:

A. Test animals:	- The number of test animals allocated to each exposure group was somewhat limited (*i.e.*, 4 rats per group at each sacrifice time). Guidelines for acute toxicity testing generally recommend use of 10 rats per exposure group. + The principle characteristics of test animals (*e.g.* specie, age, sex, pre-test health) were adequately reported. + The weight variation of animals was well below that suggested by OECD guidelines. - Only male rats were exposed in this trial. + The source of the animals was recorded. + A two-week acclimation period was allowed. - Rats are obligate nasal breathers, hence, nasal injuries may have been observed at lower levels than would have occurred if another specie had been used in the experiment.
B. Exposure conditions:	+ A whole body chamber was used. - It was not stated whether chambers were equilibrated before or after animals were placed inside. This could potentially alter the duration of exposure. +/- Since the exposure system could only expose three groups in a single "run", two independent "runs" , each with a separate control group, were performed over 2 days. + The exposure device was thoroughly described. + The exposure chamber was maintained at negative pressure in compliance guideline recommendations. + The actual gas concentrations were recorded. - Humidity and temperature during exposure were not recorded.
C. Housing/Feeding:	+ Animals were housed individually, therefore, clear observation of each animal was possible. + Commercial pelleted rat feed and water were fed *ad litum* to test animals in accordance with guideline recommendations. - Feeding restrictions imposed during exposure were not noted. +/- The material from which cages were constructed was noted. However, the dimensions of these cages were not included in their description. + Animals were housed in a room where environmental conditions were strictly controlled (*e.g.* 22+/-C; 30 to 70% humidity; monitored photoperiod)

D. Equipment:	- Monitoring devices employed were not described. + The gas delivery system was adequately described.
E. Procedural:	+ Animals were acclimatized for two weeks prior to exposure. +/- Rats were randomly allotted into exposure groups. However, the method of randomization was not noted. + Control rats were also placed within chambers to account for physiological responses associated with stress. - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- Data pertaining to the anatomic distribution of lesions in one control rat were missing. - Individual data were not provided for each test animal, hence, independent assessment of findings was not possible.
G. Data analysis:	- It was not noted whether the pathologists/histo-technicians examining the histological slides were blinded to exposure conditions.
H. Interpretations:	- Comparisons with previous studies were hampered by variations in sector labeling between laboratories.

Review - Summary:

Discussion of findings:

Exposure of male rats to 400 ppm of H_2S for 4 hours resulted in well-defined and consistent histopathological lesions within the nasal passages. Lesions were generally confined to the middle areas of the nasal passages. The rostral and caudate areas were largely unaffected by exposure. The lesions were characterized by necrosis and exfoliation of the respiratory and olfactory epithelial cells, but not the squamous epithelial cells. Within 44 hours post-exposure, the respiratory epithelial cells were in a reparative state; however, the olfactory cells remained damaged. No obvious changes in the integrity of the nasal passages were evident following exposure to 10 or 200 ppm of H_2S.

The cellular responses noted at 400 ppm were not accompanied by any obvious signs of clinical toxicity, apart from transient lethargy and epiphoria.

Interpretation of the significance and relevance of the study findings to the human condition should take into consideration the following:

- The nasal lesions were confined to the rats exposed to 400 ppm H_2S. No morphological changes in the nasal passages were noted among the rats exposed to 10 or 200 ppm, despite the vulnerability of the nasal cavity to irritant gases.
- Distinct variations in the responsiveness and adaptive capacity of the cellular linings was obvious. The squamous epithelium was unaffected by treatment, irrespective of exposure concentration. The respiratory epithelium was affected at 400 ppm, but showed evidence of repair within 44 hours post-exposure. The olfactory epithelium also was affected at 400 ppm, but remained damaged even 44 hours after exposure.
- Rats are obligate nasal breathers. Accordingly, under identical exposure conditions, the nasal passages of rodents are likely to be exposed to a higher dose of H_2S compared to humans.
- No clinical signs of toxicity were produced by the exposures to 10 or 200 ppm of H_2S. Only mild, transient signs were evident at 400 ppm.

Confidence index:

The confidence index of the study was judged to be moderate. In general, the design, conduct and reporting of the study were adequate for the purposes of the investigation. Minor deficiencies did detract from confidence in the experimental findings, and included:

- Use of limited number of test animals (*i.e.*, 4 per group per sacrifice interval).
- Use of the male sex only.
- Failure to conduct all exposures concurrently (due to limitations in equipment design).

Review – Confidence index ranking:

Moderate

Document Review

Author:	O'Donoghue, JG	**Internal ID:**	255
Title:	Hydrogen sulphide poisoning in swine.		
Year:	1961		
Abstract:	*"The exposure of young swine and rabbits to varying concentrations of hydrogen sulphide indicated that toxicity was related more to the concentration of the gas than to the length of time of exposure. Sudden exposure may reduce the minimum lethal concentration. No chronic effects were observed in animals surviving exposures as great as 1000 P.P.M. of the gas.* *It is unlikely that hydrogen sulphide poisoning would occur in domestic animals under conditions other than those that have been responsible for such fatalities in man; that is sudden exposure to gas concentration of 400 P.P.M. or greater.* *A confirmed diagnosis would have to be based on a known exposure. Pathology and toxicological examination of tissues or organs will not supply confirmatory evidence."*		
Objective:	To assess the symptoms observed in pigs and rabbits following to H_2S exposure under controlled conditions. Exposure concentrations ranged from 50 to 1200 ppm, for varying times. In many cases, exposure concentrations were adjusted upward over defined periods and exposures continued until clinical signs began severe and the animals' health was seriously compromised. The present assessment was concerned primarily with the symptoms reported at the lower exposure levels (*i.e.*, <100 ppm).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
(a) **50-100**ppm (b) 250-1000ppm (c) 400ppm (d) 350-1200ppm (e) 250-970ppm (f) 500-1050ppm (g) **50** ppm (h) 1000 ppm	(a) 2 hr (b) 2hr and 10 min (c) 1 sec (d) 44 min (e) 3 hr and 50 min (f) 36 min (g) 16 hrs (h) momentary	(a) Pig (b) Pig (c) Pig (d) Pig (e) Pig (f) Pig (g) Rabbit (h) Rabbit	Not specified	Not specified	Not specified	(a) 1 (b) 1 (c) 1 (d) 1 (e) 1 (f) 1 (g) 3 (h) 3	Not specified	50ppm	250ppm

Observations:

- Experiment (a):
  - No reported symptoms

- Experiment (g):
  - No reported symptoms

- General symptoms observed in experiments (a) to (g) in order of appearance:
  - Discomfort
  - Slight eye irritation
  - Salivation

  - Semicomatose state
  - Muscular spasms
  - Shallow breathing
  - Cyanosis
  - Death

- Post-exposure:
  - No reported after effects.

NB: In the majority of experiments, the exposure concentration was progressively adjusted upward over the range specified.

*Statistically significant

Author's Conclusions:

- *"The exposure of young swine and rabbits to varying concentrations of hydrogen sulphide indicated that toxicity was related more to the concentration of the gas than to the length of time of exposure"*
- *"Sudden exposure may reduce the minimum lethal concentration."*
- *"No chronic effects were observed in animals surviving exposures as great as 1000 P.P.M. of the gas."*
- *"A confirmed diagnosis would have to be based on a known exposure. Pathology and toxicological examination of tissues or organs will not supply confirmatory evidence."*

Review – Assessment:

A. Test animals:	- Only a single animal was exposed in six of the seven experiments. The exception was Experiment (g) in which 3 rabbits were exposed. The number of test animals was limited and did not satisfy guideline recommendations. - The breed, age and sex of exposed animals were undefined. - The pre-test health status of the animals was not indicated. - The breeding facility from which the animals were obtained was not noted.
B. Exposure conditions:	- The source of the H_2S gas was not indicated. +/- A *"titrolog instrument"* reportedly monitored the actual gas concentration continuously, but details concerning the instrumentation and readings were not provided - The animals were placed within the exposure chamber before equilibration of the gas, thereby, reducing control over exposure conditions. - The duration of exposure was uncertain. There was inconsistency within the report as to actual exposure times. +/- The mode of administration was whole body. - Airflow, temperature and humidity within the exposure chamber were not monitored.
C. Housing/Feeding:	- No information pertaining to housing conditions was provided (*i.e.*, temperature, humidity and photoperiod). - The type of feed and feeding schedule were not defined. - No details respecting water supply were given.
D. Equipment:	- Information respecting the exposure chamber and gas delivery system was lacking. The chamber was described only as *"a specifically designed chamber"*. Details concerning construction, dimensions, airflow regulation, venting *etc.* were not available. No description of the gas delivery system was provided.

	- Target concentrations evidently were monitored with a *"titrolog instrument"*, but details respecting the instrumentation and readings were lacking.
E. Procedural:	- No acclimation period or pre-test conditions (*i.e.*, quarantine, disease treatment, etc) were specified. - It was unclear whether test animals were randomly assigned to study. - It was stated that *"control animals were used throughout"*. However, information pertaining to the size of the control group, the characteristics of the control animals and the manner in which these animals were handled was lacking. - The post-exposure observation period was not specified. - Necropsy procedures were not well defined. - General experimental organization was evidently lacking since some exposures were performed *"accidentally"*. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	- It was unclear whether the nature and severity of symptoms was graded against an explicitly defined scale. - The data for control animals were not reported.
G. Data analysis:	- No statistical analyses were conducted.
H. Interpretation:	- In most instances, exposure concentrations were outside the range of interest (*i.e.*, 0-100 ppm). - Control of experimental conditions was generally lacking, thereby confounding the interpretation of findings.

Review - Summary:

Discussion of findings:

Exposure to 50ppm H_2S for up to 16 hours resulted in no observable symptoms in the exposed pig or rabbits. Higher exposure concentrations (>250 ppm) produced multiple signs of toxicity ranging from general distress through muscular weakness and in-coordination to respiratory distress, followed by unconsciousness and death. No after-effects were evident among animals that survived the exposures.

Interpretation of the significance of the study findings should take into consideration the following:

- The majority of exposure concentrations were in excess of 100 ppm.
- At exposure concentrations up to 100 ppm, no clinical signs of toxicity were reported in either pigs or rabbits. Exposures at these lower concentrations lasted from 2 to 16 hours.
- The experimental design was deficient in several respects when compared to guideline recommendations. However, as a "pilot" study, it did provide some useful information concerning the dose-responsiveness of clinical symptoms, especially at higher exposure concentrations. In addition, information respecting onset, type, severity and duration of symptoms was reported.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of very limited numbers of test animals in any given exposure "trial".
- Lack of detail concerning exposure conditions, the exposure chamber, and gas delivery system.
- Inadequate description of gas monitoring system and confirmation of reported gas concentrations.
- Complete lack of detail with respect to control animals.
- Apparent lack of control of exposure equipment, with reference to two "accidental" exposures over the course of only 7 experiments.
- Lack of detail concerning animal housing and husbandry.
- Lack of description of follow-up procedures and assessment of animal health post-exposure.
- Lack of description of necropsy procedures.

Review – Confidence index ranking:

Low

Document Review

Author:	Rogers,RE; Ferin, J	**Internal ID:**	269
Title:	Effect of hydrogen sulfide on bacterial inactivation in the rat lung		
Year:	1981		
Abstract:	*"This study evaluated the effect of a low concentration of hydrogen sulfide on the rat antibacterial defense system. Rats exposed to 45 ppm hydrogen sulfide (threshold limit value for hydrogen sulfide = 10 ppm) for 4 or 6 hr exhibited a significant ($p<.01$) reduction in the inactivation of viable staphylococci deposited in the lungs during a bacterial aerosol challenge. Pre-exposure of rats to 46 ppm hydrogen sulfide for 2 hr, however, did not alter intrapulmonary staphylococcal inactivation. We hypothesize that impairment of the alveolar macrophage is the basis of these findings."*		
Objective:	To investigate the effects of low levels of H_2S exposure on the mammalian antibacterial defense system following a bacterial aerosol challenge using adult rats.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 or 45 ppm (Whole body)	Single exposure lasting: (a) 2hr (b) 4hr (c) 6hr	Rat	Long-Evans	Not specified	Male	3-5 rats per exposure group and time.	Not specified	45ppm for 2-hr	45ppm for 4-hr

Observations:

- Inactivation of viable staphylococci:
  - 2-hr H_2S pre-exposure:
    - Sacrificed immediately: Number of colony forming units (CFU) identical to control.
    - Sacrificed 3-hr post-exposure: 55% higher CFU than control
    - Sacrificed 6-hr post-exposure: CFU identical to control.
  - 4-hr H_2S pre-exposure:
    - Sacrificed immediately: CFU identical to control.
    - Sacrificed 3-hr post-exposure: 24% higher CFU than control.
    - Sacrificed 6-hr post-exposure: 566% higher CFU than control. (*i.e.*, 6.5-fold greater percent)*
  - 6-hr H_2S pre-exposure:
    - Sacrificed immediately: CFU identical to control.
    - Sacrificed 3-hr post-exposure: 237% higher CFU than control
    - Sacrificed 6-hr post-exposure: 5100% higher CFU than control (i.e., 52-fold greater percent)*

- Clinical observations: None recorded.

- Histopathology: No evidence of pulmonary edema in lungs.

*Statistically significant

Author's Conclusions:

- *"Rats pre-exposed to 46ppm H_2S for 2hr…were not significantly different than their corresponding controls."*
- *"…4hr H_2S group had a….6.5-fold greater percent CFU remaining at the 6hr sacrifice"*
- *"…6-hr pre-exposed H_2S group had a…52-fold greater percent of CFU remaining at the same sacrifice time."*
- *…at an average concentration 4.5 times the TLV, H_2S was found to significantly reduce the inactivation of viable staphylococci in rat lungs…the magnitude of this effect increased with increasing duration of exposure to H_2S."*
- *"Experimental evidence presented here … implicates the alveolar macrophage in the H_2S effect. Impairment of the function of the alveolar macrophage therefore appears to be the basis of the H_2S effect".*
- *"In summary, a low level of H_2S significantly impaired the antibacterial system of the rat."*

Review – Assessment:

A. Test animals:	- Number of rats was limited. Only 3 rats per exposure group and sacrifice time were used. Guideline recommendations generally require 10 rats (*i.e.*, 5/sex) per group as part of conventional toxicity studies. +/- Characteristics of test animals (*e.g.* specie, strain, sex and weight) were provided; however, information pertaining to the age, pre-exposure health status and source of animals was lacking. - Only male rats were exposed. - The weight variation of test animals (29%) was outside the OECD recommended range.
B. Exposure conditions:	+/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure. + The actual (nominal) gas concentrations were recorded and analytically confirmed. The concentration of H_2S within the chamber was continuously monitored. + The precise duration of exposure was defined. - The temperature, humidity, airflow and oxygen content of the exposure chambers were not noted.
C. Housing/Feeding:	+ The material from which cages were constructed was noted (*i.e.*, stainless steel wire mesh cages). + Test animals were individually housed. +/- Rats were fed a standard laboratory diet and water *ad libitum*, but the sources of feed and water were not specified. - The environmental conditions (*i.e.*, temperature, humidity, photoperiod) of the housing room were not noted.
D. Equipment:	+ Rats were exposed to H_2S in a $1.2m^3$ Rochester chamber. +/- A full description of the device used to monitor concentration was noted, but no mention of other devices to monitor other environmental parameters. - The gas delivery system was not described.
E. Procedural:	- No indication that test animals were acclimated to the laboratory environment prior to exposure per guideline recommendations. - Random allocation of animals to exposure groups was not indicated. +/- Groups of control animals exposed to room air were included as part of the study. - It was unclear whether controls were also placed within the exposure chamber to account for physiological responses associated with the stress. - Unclear whether technicians and handlers were blinded to exposure conditions was not stated.

	- No indication that animals were sacrificed and posted randomly. +/- Some evidence that lungs were subject to histopathological examination, but no details supplied. - Conventional measures of toxicity (*e.g.*, clinical signs, body weights, gross pathology) were not included as part of the study. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	+ CFU analyses were completed in triplicate - Individual data were not provided for each test animal, thereby, limiting independent assessment of the findings.
G. Data analysis:	+ Differences between means were analyzed for significance using Student's *t* test for small unpaired samples - Values in table 2 had notably large standard deviations. - Confidence intervals were not reported.
H. Interpretation:	+ The author introduced several hypothesis to explain the higher CFU in H_2S exposed rats.

Review - Summary:

Discussion of findings:

Exposure to 45ppm H_2S for a period of 2 hours resulted in no significant changes in the antibacterial defense capacity of exposed rats. However, following 4 hours and 6 hours H_2S exposure, the inactivation of *Staphylococcus epidermidis* was significantly reduced. The mechanism by which H_2S exposure resulted in the noted 6.5-fold and 52-fold respective reductions is not known. The authors postulated that the persistent viable bacteria in the lungs of exposed rats can be attributed to altered macrophage function. Impaired mucociliary clearance and bacterial replication were both addressed and dismissed as possible causes.

Interpretation of the significance of the study findings should take into consideration the following:

- The study was performed with a very limited number of test animals, contributing to large standard deviations surrounding the bacterial colony counts. However, despite the inter-animal variability, the differences in viable bacteria counts between the treated and control groups still achieved statistical significance, thereby strengthening the significance of the findings. Interpretation might have been improved through use of larger group sizes.
- The dose-responsiveness of the observed changes in the antibacterial defense system could not be determined owing to the use of a single exposure concentration only. Interpretation of the significance of the findings could have been improved through use of graded exposure levels.
- The handling of the control group of animals was not entirely clear. The report states only that the control animals "*were exposed to room air in each experiment*". It is not certain as to whether the control rats were exposed to the room air within the exposure chamber or simply within the animal housing room. Since stress has been shown to impair the antibacterial defense system of the lung, the reported findings could be misleading if the control animals had been exposed under different conditions than the treated rats (*i.e.*, confinement in the exposure chamber would likely be accompanied by stress).
- The change in the antibacterial defense system became progressively more severe with increased exposure time, demonstrating consistent time-responsiveness and strengthening the significance of the findings.
- The relevance of the findings to the human condition should respect the fact that rats are obligate nose breathers, and the penetration of H_2S to the alveolar regions of the lung in the rat may differ from the human owing to possible capture of the gas as it is swept across the nasal passages. However, at the same time, the increased ventilation rate of the rat may increase the overall H_2S burden on the respiratory tree compared to humans.
- The findings from the study are consistent with "real-world" experience, and provide a reasonable explanation to account for the secondary pneumonia that frequently follows H_2S poisoning. Impairment of the bacterial defense system in the presence of pulmonary edema could provide an optimal environment for the development of pneumonia. The association could have been strengthened by use of graded exposure concentrations, particularly higher exposure levels that might have induced pulmonary edema.

Confidence index:

The confidence index of the study was judged to be moderate. Most elements of study design, conduct and reporting were judged to be adequate for the purposes of the investigation. However, greater confidence in the study findings and conclusions could have been achieved by:

- Use of larger group sizes of test animals.
- Use of both sexes of rats.
- Use of graded exposure concentrations.
- Clearer description of animal handling and exposure conditions.
- Added description of the exposure chamber, including the gas delivery system.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Saillenfait, AM; Bonnet, P; de Ceaurriz, J	**Internal ID:**	278
Title:	Effects of inhalation exposure to carbon disulfide and its combination with hydrogen sulfide on embryonal and fetal development in rats.		
Year:	1989		
Abstract:	*"Pregnant rats were exposed to 0, 100, 200, 400 or 800ppm of carbon disulphide (CS_2), 100ppm of hydrogen sulfide (H_2S) alone or in combination with 400 and 800ppm CS_2 6h/d during 6-20 of gestation.* *Maternal reproduction and fetal parameters were evaluated on gestational day 21. Treatment with 100 or 200ppm CS_2 or with 100ppm H_2S caused no maternal toxicity or adverse effects on the developing embryo or fetus. Exposure to 400 or 800ppm CS_2 resulted in a low incidence of clubfoot and in a significant reduction of maternal weight gain. Significant increases in unossified sternebrae occurred at 800ppm CS_2 and reduction of fetal body weight at 400 and 800ppm CS_2. The latter effect was enhanced by combination with 100ppm H_2S.* *These results support the conclusion that at levels of exposure associated with maternal toxicity, CS_2 leads to an increase in incidence of club foot and to fetal toxicity which is enhanced by simultaneous exposure to H_2S."*		
Objective:	To examine the effects of inhaled CS_2 and H_2S, either alone or in combination, on embryonal and fetal development in the rat. [N.B. The study was comprised of five separate experiments. Only experiment (a) and (c) complied with the inclusion criteria determined for the present review. Experiment (a) was a pilot study to establish the maternally toxic dose of H_2S].		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
(a) 0; **50**; **100**; 150ppm H_2S (b) 0; 100; 200; 400; 800ppm CS_2 (c) 0; **100**ppm H_2S (d) 0; **100**ppm H_2S + 400ppm CS_2 (e) 0; **100**ppm H_2S + 800ppm CS_2	Repeated/ 6 hours per day from day 6 to 20 of gestation.	Rat	Sprague-Dawley	Not specified.	Female	(a) 33 (b) 133 (46 control; 87 treated) (c) 69 (46 from (b) control; 23 treated) (d) 69 (46 from (b) control; 23 treated) (e) 69 (46 from (b) control; 23 treated)	Not specified.	50ppm H_2S (?)	100ppm H_2S

Observations:

- H_2S exposure alone: (Data from experiment (a) unless otherwise noted)
  - Incidence of pregnancy: No significant change
  - Body weight gain of dams:
    - **50**ppm: 0.8% increase
    - **100**ppm (Experiment a): 13% decrease
    - **100**ppm (Experiment c) 2: 8% decrease
    - 150ppm: 21% decrease*
  - Absolute weight gain of dams:
    - **50**ppm: No change

  - **100**ppm (Experiment a): 45% decrease*
  - **100**ppm (Experiment c): 20% decrease
  - 150ppm: 150% decrease*
- Mean implantation sites per litter:
  - **50**ppm: 11% increase
  - **100**ppm (Experiment a): 21% increase*
  - **100**ppm (Experiment c): 0.6% increase
  - 150ppm: 15% increase*
- Mean resorption sites per litter:
  - **50**ppm: No change
  - **100**ppm (Experiment a): 14% decrease
  - **100**ppm (Experiment c): 17% increase
  - 150ppm: 43% decrease
- Mean dead fetuses per litter (Experiment a and c): No change.
- Mean live fetuses per litter:
  - **50**ppm: 9% increase
  - **100**ppm (Experiment a): 21% increase*
  - 100ppm (Experiment c): No change
  - 150ppm: 18% increase*
- Mean fetal body weight:
  - **50**ppm: 4% decrease*
  - **100**ppm (Experiment a): 7% decrease*
  - **100**ppm (Experiment c):
    - Males: 0.5% increase
    - Females: 0.96% decrease
  - 150ppm: 7% decrease*
- External anomalies (Experiment a and c): No change.
- Fetal sex ratio (Experiment c): No change.
- Soft tissue abnormalities (Experiment c): No change.
- Skeletal abnormalities (Experiment c): No change.

*Statistically significant

Author's Conclusions:

- *"Treatment with 100 ppm H_2S caused no maternal toxicity or adverse effects on the developing embryo or fetus".*
- *"Exposure to 50ppm H_2S had no significant effect on any of the parameters explored."*
- *"Simultaneous exposure to 100ppm H_2S and either 400 or 800ppm CS_2 weakly enhanced maternal toxicity induced by 400 or 800ppm CS_2 alone. Lower fetal body weight was the only parameter indicating fetal toxicity to be significantly potentiated by combinations of CS_2 with H_2S."*
- *"Exposure to 100ppm H_2S in combination with 400 or 800ppm CS_2 only enhances fetal toxicity of CS_2 administered alone."*

Review – Assessment:

A. Test animals:	+ The number of dams allocated to each exposure group was clearly indicated and complied with guideline recommendations for the conduct of developmental toxicity studies. +/- Most of the principle characteristics of test animals (*e.g.*, specie, sex, weight variation and source) were recorded. Omissions included age and pre-test health status. + Weight variation of dams at study initiation was less than 20%, in accordance with guideline recommendations..
B. Exposure conditions:	+ The source of the H_2S gas was indicated. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibrium period. - The total animal displacement in the chambers was not reported. + Environmental conditions were controlled (*e.g.*, temperature, pressure, humidity and airflow) and values were within guideline recommendations. + Variation in measured H_2S concentration was no more than 5% from the corresponding nominal concentration. + The analytical concentrations of H_2S were determined and reported. + The exposure regimen was justified (*i.e.*, the exposure concentration of 100 ppm was selected on the basis of a pilot study).
C. Housing/Feeding:	+ Details concerning animal housing (*i.e.*, temperature, humidity and photoperiod) were provided and complied with OECD recommendations. - Details concerning caging were lacking. - It is unclear as to whether the rats were housed continuously in the exposure chamber or introduced daily into the chamber during the 6h exposure period only. - Bedding and nesting materials were not specified. + Animals were allowed to eat and drink *ad libitum* except during the exposure period, consistent with guideline recommendations. +/- The source of the feed was reported, but the type was not stated. - Water supplied was tap water. - How animals were caged (*i.e.*, individually or grouped) was not recorded.
D. Equipment:	+ Description of the exposure chamber (200 L stainless steel) was adequate but minimal. A schematic would have been beneficial. +/- H_2S and CS_2 concentrations were determined spectrophotometrically. - The manner by which temperature, humidity and airflow were determined within the exposure chamber was not stated.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. +/- Gravid females were randomly assigned to exposure groups, but the method of randomization (*e.g.*, table of random numbers, computer generated, *etc.*) was not indicated. +/- A control group was included for each experiment. Control animals were exposed to room air in a similar flow chamber. Unfortunately, the potential for air contamination was not assessed. - Unclear whether technicians or handlers were blinded to exposure conditions. + Conventional measures of toxicity were included as part of study. - No indication that the study was performed under Good Laboratory Practice (GLP) conditions. + Multiple exposure concentrations were measured, thereby permitting examination of dose-responsiveness. + The method by which animals were sacrificed was reported.

F. Data collection:	- Individual data were not provided for each test animal, thereby limiting the independent assessment of the findings. +/- Data from control animals from the separate experiments were averaged. Before collapsing the data, the researchers did evaluate, by some undefined means, the statistical validity of combining the findings.
G. Data analysis:	+/- The litter was considered the experimental unit for analysis. + Statistical analyses were conducted using Yate's corrected chi-square test, Student's t test, or the Mann-Witney U-test. - Confidence intervals were not provided.
H. Interpretations:	- Identical exposure concentrations (100ppm H_2S) yielded different results between Experiment (a) and Experiment (c). - For multiple parameters, the standard deviations were notably large.

Review - Summary:

Discussion of findings:

Exposure of Sprague-Dawley rats to graded concentrations of H_2S (0, **50**, **100**, 150ppm) resulted in a dose-related change in mean implantation sites per litter and in both maternal and fetal body weight. The number of live fetuses was significantly increased in rats exposed to **100** and 150ppm H_2S, but the degree of response was not related to dose. No significant changes were observed in any of the remaining measures of toxicity assessed (*e.g.*, number of resorption sites, number of dead fetuses, external fetal anomalies, soft tissue anomalies, skeletal anomalies and fetal sex ratio). Changes observed were confined to the higher exposure groups. No significant changes were observed following exposure to 50ppm H_2S except a 4% decrease in mean fetal body weight.

Interpretation of the significance of the study findings should take into consideration the following:

- Although the study investigators concluded that exposure to 100 ppm of H_2S was without effect on maternal parameters, the conclusion is not entirely supported by the experimental findings. Specifically, in Experiment (a), a dose-related reduction in maternal body weight gain during gestation was evident among the dams exposed to 100 ppm (*i.e.*, 15% decrease) and 150 ppm (*i.e.*, 21% decrease) relative to the corresponding control value. The differences were statistically significant. Similarly, in Experiment (b), the absolute weight gain of the dams exposed to 100 pm was reduced relative to controls (*i.e.*, by 8%); however, the difference was not statistically significant. The changes are suggestive of mild maternal toxicity at 100 ppm.
- In addition, despite the authors' contention that exposure to 100 ppm was without effect on fetal parameters, the study findings might suggest otherwise. Specifically, in Experiment (a), a dose-related trend toward reduced fetal body weights at the time of delivery was apparent, with reductions of 4 %, 9% and 9% relative to controls noted at exposure concentrations of 50 ppm, 100 ppm, and 150 ppm of H_2S, respectively. The differences were statistically significant at the two higher exposure levels. A similar difference in body weights was not evident in Experiment (b). Owing to the lack of consistency in findings between the two experiments, it is difficult to assign significance to the changes in fetal body weights observed in the first study; however, the change is perhaps suggestive of fetotoxicity.
- The study was directed primarily at determining the effects of carbon disulphide, and not hydrogen sulphide, on maternal and fetal health

Confidence index:

The level of confidence that could be assigned to the study findings and conclusions was judged to be moderate to high. The design and conduct of the study was generally consistent with guideline recommendations, with no obvious deficiencies.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Savolainen, H; Tenhunen, R; Elovaara, E; Tossavainen, A	**Internal ID:**	280
Title:	Cumulative biochemical effects of repeated subclinical hydrogen sulfide intoxication.		
Year:	1980		
Abstract:	*"Adult female NMRI mice exposed four times to 100 ppm hydrogen sulfide vapour for 2 h at 4-day intervals showed increasing inhibition of the cerebral cytochrome oxidase activity. Cerebral RNA decreased significantly after the fourth exposure. This change was accompanied by the reduced orotic acid uptake in the RNA fraction. At the same time, 2',3'-cyclic nucleotide 3'-phosphohydrolase activity used as a marker for glia increased. Acetylcholine esterase activity remained unchanged. The initial exposures also caused an increase in the superoxide dismutase activity and an increase in the glutathione concentration. The latter effects were abolished in the third and fourth exposures. The present data seem to indicate that the biochemical effects of repeated subclinical hydrogen sulfide intoxications are cumulative."*		
Objective:	To investigate the effect of repeated subclinical hydrogen sulfide intoxication on the biochemical profile of the mouse brain.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 ppm; 100ppm	Two hour exposure, repeated once every four days for up to 16 days	Mouse	NMRI	Adult	Female	28. (20 treated; 8 controls). Treated animals were divided into groups of 5 and sacrificed at 4-day intervals following exposure.	Not specified.	NA	100ppm

Observations:

- Behavioral observations:
  - Excitation during initial phase of each exposure period
  - None of the mice died
  - No clinical signs of hydrogen sulfide toxicity

- Cerebral protein content: No significant change

- Cerebral glutathione content:
  - Single 2h exposure: 0.5% decrease
  - Two 2 hour exposures: 10% increase*
  - Three 2 hour exposures: No change
  - Four 2 hour exposures: 4% decrease*

- Cerebral RNA content:
  - Single 2 hour exposure: No change
  - Two 2 hour exposures: 4% increase
  - Three 2 hour exposures: No change
  - Four 2 hour exposures: 7% decrease*

- Cerebral orotic acid uptake:
    - Single 2 hour exposure: 6% decrease
    - Two 2 hour exposures: 12% decrease*
    - Three 2 hour exposures: No data
    - Four 2 hour exposures: 17% decrease*

- Cerebral cytochrome aa_3 inhibition:
- Single 2 hour exposure: 4% inhibition
    - Two 2 hour exposures: 9% inhibition
    - Three 2 hour exposures: 11% inhibition
    - Four 2 hour exposures: 15% inhibition

- Lysosomal acid proteinase activity:
    - Single 2 hour exposure: 2% decrease
    - Two 2 hour exposures: 1% increase
    - Three 2 hour exposures: 3% increase
    - Four 2 hour exposures: 5% decrease*

- Cerebral superoxide dismutase activity:
    - Single 2 hour exposure:10% increase*
    - Two 2 hour exposures: 4% increase
    - Three 2 hour exposures: 0.6% decrease
    - Four 2 hour exposures: 1.2% decrease

- Cerebral 2',3'-cyclic nucleotide 3'-phosphohydrolase:
    - Single exposure: No change
    - Two 2 hour exposures: 5% increase
    - Three 2 hour exposures: 10% increase
    - Four 2 hour exposures: 10% increase*

- Cerebral acetylcholine esterase activity: No significant change

*Statistically significant

Author's Conclusions:

- *"The cause of biochemical effects...can be very well explained by the cumulative inhibition of the cytochrome oxidase...results seem to point to only a partial resolution of the inhibition between the exposures...a limiting factor in the recovery could be, in fact, the rate of synthesis of new haem."*
- *"...lysosomal proteases assume new steady states very rapidly according to the changes in protein synthesis...The reduced proteolysis could be thereby explained, and it would agree with the reduced RNA precursor uptake."*
- *"Hypoxic episodes are also known to give rise to increased formation of peroxides...which could explain the initial increase in the superoxide dismutase activity."*
- *"The increase in glutathione concentration after the second exposure and gradual decrease in the successive exposures can be thought to indicate progressive failure in the maintenance of the cellular redox equilibrium."*
- *"Glial cells seem to be vulnerable to prolonged hypoxia....2',3'-cyclic nucleotide 3'-phosphohydrolase activity displays an increasing trend in the present study. This enzyme has been used as a marker for the myelin-forming glia."*
- *"...acetylcholine esterase activity of primarily neuronal origin remained unaltered."*
- *"...data seem to indicate that the biochemical effects of repeated subclinical hydrogen sulfide intoxications are cumulative."*
- *"Similar inhibition of cytochrome oxidase might take place also in the human brain which could very well lead to the increased headache frequency, increased fatigue and slow deterioration of mental functions sometimes seen in chronic human hydrogen sulfide toxicity"*

Review – Assessment:

A. Test animals:	- Number of test animals was limited (*i.e.*, 5 per treatment group at each sacrifice time). Guidelines for subacute toxicity testing generally recommend 10-20 rodents per group (5-10 per sex), with numbers increased if interim sacrifices are planned. +/- Most of the principle characteristics of test animals (*e.g.*, specie, strain, sex, age, weight variation) were reported but the pre-test health of animals and the breeding facility from which they were obtained was not defined. + Weight variation of animals was within OECD guidelines. - Only the female sex was tested. Guidelines typically recommend that both sexes be tested.
B. Exposure conditions:	- The source of the H_2S gas was not indicated. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibrium period. - The total animal displacement in the chambers was not reported. - Whether H_2S concentration, oxygen content, temperature, pressure, humidity, and photoperiod were monitored throughout exposure was not reported.
C. Housing/Feeding:	- Details concerning animal housing (*i.e.*, temperature, humidity, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type, and dimensions) was not provided. - Bedding material was not specified. - The type and source of feed supplied was not reported. The feeding schedule was also omitted. - Details concerning the water supply were lacking.
D. Equipment:	+/- Descriptions of the exposure chamber and gas delivery system were minimal as they were described previously in Elovaara *et al.* (1978) Study ID-150. - Equipment used to monitor environmental conditions within the chamber was not described.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided.

	- Absence of data pertaining to the randomization of test animals and assignment to test groups. +/- Only a single control group was included. Accordingly, most treatments were performed in the absence of a concurrent control group. - Other than a precursory assessment of behavior, conventional measures of toxicity (*i.e.*, body weights, clinical pathology, organ weight, gross pathology, histopathology) were not included. - A single exposure concentration was tested, thereby, precluding any examination of dose-responsiveness - Whether biochemical analyses were conducted in replicate was not specified. - Unclear whether technicians and handlers were blinded to exposure conditions. + All biochemical assays were referenced. + Rationale for the exposure regimen was provided. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	- Individual data were not provided for each test animal, thereby limiting the independent assessment of the findings. - Information pertaining to which cerebral hemisphere was sampled was lacking.
G. Data analysis:	+ Data was statistically evaluated with variance analysis. - Confidence intervals were not provided.
H. Interpretations:	- Results for cytochrome oxidase activity were expressed in terms of percent inhibition relative to control values. All other enzyme activities were expressed in terms of absolute units. No explanation was provided to account for the difference. + The author provided a biologically plausible explanation for observed results in the discussion section, but their extrapolation of the findings to humans was not substantiated by empirical evidence.

Review - Summary:

Discussion of findings:

Mice exposed to 100ppm of H_2S for 2 hours at four-day intervals for up to 16 days reportedly showed changes in a number of metabolic and enzymatic parameters in cerebral tissue. The changes included progressive inhibition of cytochrome oxidase activity, reduction in RNA synthesis, increased superoxide dismutase activity, increased glutathione concentration and increased activity of 2', 3'-cyclic nucleotide 3'-phosphohydrolase. The authors ascribed the changes to the cumulative inhibition of cytochrome oxidase, with attendant cerebral hypoxia. It was postulated that the four-day interval between successive exposures to H_2S did not allow for complete recovery of cytochrome oxidase function. The resulting cerebral hypoxia gave rise to increased formation of peroxides (consistent with the increased superoxide dismutase activity), altered cellular redox potential (consistent with the increased glutathione levels), and reduced protein synthesis (consistent with the reduction in RNA synthesis). No changes in cerebral protein levels or acetylcholinesterase activity were found. Apart from some initial "excitement" at the initiation of each exposure period, the changes were not accompanied by any clinical signs of intoxication.

Interpretation of the significance of the study findings should take into consideration the following:

- The majority of changes in metabolic and enzymatic parameters were relatively modest, with differences from control values being routinely less than 10 percent. In only some instances did the differences achieve statistical significance.
- For many of the parameters, the changes were not consistent across the entire treatment schedule, with the differences from control values changing direction at each treatment interval. For example, glutathione content was marginally *decreased* upon the first treatment, *increased* upon the second treatment, *unchanged* upon the third treatment, and then *decreased* upon the fourth and final treatment relative to controls. Similarly, RNA content was *unchanged* upon the first treatment, *increased* upon the second treatment, *unchanged* upon the third treatment, and *decreased* upon the fourth treatment. Finally, superoxide dismutase activity was alternately *increased* and then *decreased* upon the first and second treatments and the third and fourth treatments, respectively, relative to controls. Despite the claims of the authors, these shifts in the differences relative to control values for each of these parameters are not consistent with treatment-related effects

- If, as the authors suggest, the changes in the metabolic and enzymatic parameters result from the cumulative inhibition of cytochrome oxidase, the responses would reasonably be expected to progress with each successive treatment (*i.e.*, a progressive increase or decrease in content or activity would occur). However, as indicated above, for many of the reportedly affected parameters, the changes were not progressive, but rather fluctuated between each treatment interval. The rather random nature of the responses undermines the association that the authors claim exists between the various parameters and the inhibition of cytochrome oxidase.
- The findings did reveal a progressive increase in the inhibition of cytochrome *aa*$_3$ oxidase with each successive treatment. However, at no time did the difference from the control value achieve statistical significance. The lowest level of inhibition was marginal (*i.e.*, < 5% after the first treatment), while the highest level approached 15 percent. (*i.e.*, after the fourth treatment). It was of some interest that the activity of cytochrome oxidase was expressed as "% inhibited" relative to controls, while all remaining enzyme activities were expressed in absolute units. No explanation was provided for the difference in expression. The lack of absolute values confounded the interpretation of the results. However, it is accepted that H_2S will inhibit cytochrome oxidase.
- Changes were based on measurements made on cerebral tissue obtained from only 5 female mice at each treatment interval. Confidence in the findings would have been improved by increasing the group size, in accordance with the recommendations contained in testing guidelines for subacute toxicity studies (*i.e.*, 10-20 animals per treatment group).
- The dose-responsiveness of the observed changes could not be determined owing to the use of a single exposure concentration only. Confidence in the findings would have been improved through the use of graded exposure levels, consistent with guideline recommendations (*i.e.*, a minimum of 3 treatment levels, plus a control group).
- Despite the inhibition of cytochrome oxidase and the presumption of cerebral hypoxia, the authors reported that *"none of the treated mice died or presented clinical signs of hydrogen sulfide toxicity"*. Thus, the clinical relevance of the changes in metabolic and enzymatic parameters is questionable.
- Apart from a brief description of clinical observations, the study lacked any conventional measures of toxicity that might have improved interpretation of the observed changes in metabolic and enzymatic parameters. Greater attention to clinical observations and histological examination of the cerebral tissue may have provided supportive evidence attesting to the significance of the reported changes. In the absence of such supportive evidence, the clinical and toxicological relevance of the findings are questionable and the conclusions should be considered tenuous.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Use of only limited number of test animals.
- Use of female mice only.
- Use of a single exposure concentration only.
- Use of a single control group.
- General lack of detail respecting animal housing and husbandry.
- Lack of conventional measures of toxicity to permit better understanding of findings.

Review – Confidence index ranking:

Low to moderate

Document Review

		Internal ID:	290
Author:	Skrajny, B; Hannah, RS; Roth, SH		
Title:	Low concentrations of hydrogen sulfide alter monoamine levels in the developing rat central nervous system.		
Year:	1992		
Abstract:	*"The central nervous system in one of the primary target organs for hydrogen sulphide (H_2S) toxicity; however, there are limited data on the neurotoxic effects of low-dose chronic exposure on the developing nervous system. Levels of serotonin and norepinephrine in the developing rat cerebellum and frontal cortex were determined following chronic exposure to 20 and 75 ppm H_2S during perinatal development. Both monoamines were altered in rats exposed to 75ppm H_2S compared with controls; serotonin levels were significantly increased at days 14 and 21 postnatal in both brain regions, and norepinephrine levels were significantly increased at days 7, 14, 21 postnatal in cerebellum and at day 21 in the frontal cortex. Exposure to 20ppm H_2S significantly increased the levels of serotonin in the frontal cortex at day 21, whereas levels of norepinephrine were significantly reduced in the frontal cortex at days 14, 21, and at day 12 in the cerebellum."*		
Objective:	To investigate the effect of perinatal exposure to 20ppm or 75ppm H_2S on the monoamine neurotransmitters, norepinephrine and serotonin, in the cerebellum and frontal cortex of developing rats		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 20ppm; 75ppm (whole body)	Repeated/ 7h per day from day 5 postcoitus until day 21 postnatal	Rat	Sprague-Dawley	Not specified	Pregnant female dams. Sex of the pups that were examined was not specified.	Ten pups per exposure group at each of 3 sampling times.[1]	Timed-pregnant. Pup health was not evaluated.	NA	20ppm (?)

1. According to the paper, groups of 20 dams were exposed to either 20 or 75 ppm H_2S. A separate control group was included, but the number of control dams was not specified. At parturition, each group was culled to 6 dams. Thirty pups from each group were then selected from the available litters and sacrificed at Day 7, 14 or 21 post-partum (*i.e.*, 10 pups per group per sampling time).

Observations:

- Serotonin (5-HT) levels:
    - Frontal cortex:
        - 20ppm:
            - Day 7 post-partum: 13% decrease relative to controls
            - Day 14: 5% increase
            - Day 21: 26% increase*
        - 75ppm:
            - Day 7: 6% increase
            - Day 14: 35% increase*
            - Day 21: 57% increase*
    - Cerebellum:
        - 20ppm:
            - Day 7: 5% decrease

    - Day 14: 5% increase
    - Day 21: 10% increase
  - 75ppm:
    - Day 7: 4% decrease
    - Day 14: 31% increase*
    - Day 21: 29% increase*

- Norepinephrine (NE) levels:
  - Frontal cortex:
    - -20ppm:
      - -Day 7 post-partum: 13% decrease relative to controls
      - -Day 14: 15% decrease*
      - -Day 21: 19% decrease*
    - -75ppm:
      - -Day 7: 21% increase
      - -Day 14: 5% increase
      - -Day 21: 37% increase*
  - Cerebellum:
    - - 20ppm:
      - -Day 7: 26% increase*
      - -Day 14: 13% decrease*
      - -Day 21: 2% increase
    - - 75ppm:
      - -Day 7: 26% increase*
      - -Day 14: 30% increase*
      - -Day 21: 13% increase*

*Statistically significant

Author's Conclusions:

- *"At 75ppm H_2S, the levels of 5-HT from the cerebellum and frontal cortex of the treated animals were significantly higher ($p< 0.01$) than control levels at day 14 and 21 postnatal."*
- *"Significant changes in 5-HT levels were only observed following exposure to 20ppm H_2S at day 21 in the frontal cortex tissue."*
- *"At 75ppm H_2S, NE levels in the cerebellum of treated animals were significantly elevated above the control levels are all three test periods. The levels in the frontal cortex appeared to be significantly increase only at day 21 postnatal."*
- *"Exposure to 20ppm H_2S also appeared to alter the NE levels. In the frontal cortex NE levels were significantly reduced below control levels by days 14 and 21 postnatal. In the cerebellum, NE levels were initially increased at day 7, then decreased at day 14, and returned to control levels by day 21 postnatal."*
- *"Perhaps the most significant finding of this study is that exposure to 75ppm H_2S during development of rat CNS results in increased 5-HT and NE levels in both the cerebellum and the frontal cortex."*
- *"...increase in the 5-HT levels appears to be dose-dependent"*
- *"...Opposite effects on NE levels in the cerebellum were observed, i.e., 20ppm H_2S resulted in a depression at day 14, whereas 75ppm produced an elevation....These suggest that H_2S may alter more than one biochemical process, involved in monoamine turnover, including synthesis, release, or*

biotransformation."

- *"It should be noted that during H$_2$S exposure, monoamine levels did not return to control levels.... The present results, therefore, suggest that H$_2$S may cause irreversible changes in monoamine levels in the developing rat CNS."*
- *"It is possible, therefore, that morphological changes observed in the cerebellum may be explained by an increase in the monoamine levels during a critical stage of development. These results suggest that alterations of monoamine levels induced by H$_2$S may produce long-lasting morphological and neurochemical changes in the CNS."*

Review – Assessment:

A. Test animals:	+/- Number of treated dams on test (n=20 per group) was adequate and consistent with recommendations contained in testing guidelines for developmental toxicity studies. However, number of control dams was not specified. + The specie, strain and source of test animals were reported. - Weight variation, pre-test health status and the age of the dams was not noted. - The sex of the pups selected for examination was not indicated. - At parturition, each group was culled to 6 dams, with a maximum of 12 pups in each litter. The basis for the culling was not indicated and this type of culling represents a departure from guideline recommendations. - The number of pups examined was somewhat limited (*i.e.*, 10 pups per group for each of 3 sampling times), especially given the number of pups which theoretically should have been available for examination (*i.e.*, a minimum of 96 pups per group based on culling to 6 litters or approximately 180 to 200 pups per group if all litters had been included). .
B. Exposure conditions:	- The source of H$_2$S gas was not reported. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibration period. + Chamber was designed to permit continuous observations of animals. +/- H$_2$S concentrations, airflow (20 changes per hour), temperature, pressure, humidity, and photoperiod were monitored throughout exposure, but the results of most of these analyses were not reported. Accordingly, it was not possible to verify that the chamber conditions met guideline recommendations, nor to confirm that the target concentration of H$_2$S was achieved + H$_2$S concentrations were monitored at the animal level. +/- Dams and/or litters were evidently moved in and out of the exposure chambers daily for 7 hours from a central housing unit. There was no indication that the animals were pre-conditioned to the exposure chambers prior to treatment in order to minimize stress.
C. Housing/Feeding:	- Other than specification of the photoperiod, details concerning environmental conditions within the animal rooms were not supplied. - Details concerning caging, bedding and nesting materials were not specified. + Animals were allowed to eat and drink *ad libitum* except during the exposure period, consistent with guideline recommendations. - The source and type of feed supplied was not noted. - Details concerning the water supply were not provided.
D. Equipment:	+/- Details of exposure chamber (*e.g.*, dimensions, material, *etc.*) were provided in a companion paper (Hayden *et al.*, 1990 – Study ID 12). + The chamber allowed continuous observation of the animals, monitoring and recording of temperature, pressure, humidity, and H$_2$S concentration.

	+/- The equipment used to measure the H_2S concentration (pre-calibrated digital GFG model GMA-100 monitoring system) and airflow concentration (orifice plate) were reported, but the manner by which humidity, temperature, and oxygen levels were determined was not outlined.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. +/- Pups were randomly chosen for euthanization at each sample time, but whether dams were randomly assigned to exposure groups was not noted. Details concerning the method of randomization (*e.g.* table of random numbers, computer generated, etc.) were not supplied. - Method of culling of dams was not specified. - Method of selection of pups from available litters was not indicated. +/- Control animals were included and *"treated under similar conditions"* but without H_2S exposure. Treated and control animals were exposed simultaneously in two identical chambers. Consequently a minimum of two control groups would have been utilized. The authors subsequently, averaged control results, but this was done without reporting the values of each control group separately and the investigators did not conduct a statistical analysis to ascertain whether the averaging of these results was valid. - Unclear whether technicians and handlers were blinded to exposure conditions. +/- The weights of the pups of treated and control dams were compared, but other conventional measures of toxicity (*i.e.*, clinical observations, pregnancy outcome, litter parameters, gross pathology) were not performed. - No indication that study was performed under Good Laboratory Practice (GLP) conditions. - Only two treatment levels were tested. Guidelines for developmental toxicity studies generally recommend use of three treatment levels.
F. Data collection:	- Individual animal data were not provided, thereby limiting the independent assessment of the findings. - No indication that analyses for monoamine levels were performed in replicate. - Except for control data, results were graphically displayed and resolution was generally lacking. - Weights of the neonates reportedly did not differ between groups, but no numerical findings were provided. - The number of samples analyzed and averaged to produce the graphical results was not indicated. - The findings were presented on a pup basis only, without regard to inter-litter variability.
G. Data analysis:	+ Statistical evaluation of the data was carried out with the Student's *t* test. - Confidence intervals were not provided. - Inter- and intra-litter data were not separately analyzed.
H. Interpretations:	- Conclusions were largely based on hypothesis, without support of empirical data. - Interpretation of findings is confounded by experimental design, which evidently involved 2 separate exposure trials. - Lack of detail concerning culling methods and selection of pups for examination introduces possibility of selection bias.

Review - Summary:

Discussion of findings:

In utero and post-natal exposure of rats to 20 ppm and 75 ppm of H_2S for 7 hours per day from Day 5 post-coitus to Day 21 post-partum reportedly resulted in changes in the levels of serotonin and norepinephrine in the developing cerebellum and/or frontal cortex. The authors postulated that these changes in monoamine levels could result in long-lasting morphological and neurochemical alterations in the central nervous system.

Interpretation of the significance of the study findings is confounded by the following:

- Lack of clear dose-responsiveness, combined with inconsistency in the nature of the responses at different sampling times (*i.e.*, the patterns of response were generally inconsistent across exposure groups and/or sampling times). For example, norepinephrine levels within the frontal cortex of the developing brain were decreased relative to control values at all sampling times following exposure to 20 ppm H_2S, but increased after exposure to 75 ppm. Further, norepinephrine levels measured in the cerebellum following exposure to 20 ppm were increased relative to control values at Day 7 post-partum, decreased on Day 14 post-partum, and increased on Day 21 post-partum. In addition, serotonin levels in the cerebellum were decreased relative to controls at Day 7 post-partum and increased at Days 14 and 21 post-partum following exposure to either 20 ppm or 75 ppm H_2S. In fact, a consistent response across both exposure concentrations and sampling times was never realized. This lack of consistency renders interpretation of the findings very difficult.

- Use of unconventional culling methods and lack of description of methodology used for selecting pups for examination. The report indicated that groups were culled to 6 dams at parturition. By convention, culling usually applies only to the pups, with the number of pups per litter culled to 8 on Day 4 post-partum to avoid undue stress of the nursing mother and to minimize inter-litter variability. The culling of dams signifies the destruction and loss of litters, a practice that is generally discouraged in developmental toxicity studies. Moreover, the manner in which the dams were either removed or retained was not indicated, thereby introducing the possibility of selection bias. Similarly, no indication was provided as to how pups were selected from the available litters for examination and whether or not all litters were represented. Again, this presents the possibility of selection bias.
- Lack of conventional measures of toxicity to complement the analyses of brain monoamine levels. No clinical observations of the dams or pups were recorded. Interpretation of the meaning and significance of the reported changes in serotonin and norepinephrine levels might have been assisted by careful observation and recording of clinical signs of toxicity that could have signified neurophysiological or neurobehavioral abnormalities. Similarly, histopathological and/or histochemical analyses of the cerebellum and frontal cortex might have revealed some histological correlates of the changes in monoamine levels. Without collaborative evidence, the reported association between the shifts in brain monoamine levels and long-lasting morphological and neurochemical changes in the CNS remains conjecture only, and cannot be used to assign significance to the study findings. Further, in the absence of information respecting clinical signs, it is impossible to determine to what degree, if any, maternal stress may have been implicated in the changes in monoamine levels in the developing pups. It is conceivable that stress could alter monoamine levels; hence, observation of the dams would have permitted some indication of whether monoamine shifts were a result of direct H_2S toxicity or maternal stress. In developmental toxicity studies, less significance is generally assigned to responses in the fetuses that occur secondary to maternal stress.
- Lack of understanding of the toxicological impact of shifts in brain monoamine levels, especially given the functional reserve of the central nervous system.

The study might have been improved and interpretation of the findings eased by:

- Better adherence to conventional protocols for the study of developmental toxicity, including the inclusion of routine clinical observations.
- Better description of test methodologies, including randomization and culling methods.
- Avoidance of culling of dams.
- Assessment of findings on both a litter and fetus basis.
- Histopathological examination of the brain regions for evidence of morphological damage consistent with altered monoamine levels.
- Inclusion of an additional exposure concentration, as recommended by most guidelines, to provide better definition of dose-responsiveness.
- Inclusion of a recovery period to better gauge the likelihood of long lasting changes in the morphological architecture and/or function of the CNS caused by the shifts in monoamine levels.

Any assessment of the relevance of the study findings to the human condition should consider the nature of the exposure regimen, involving the daily exposure of the dams and/or pups for 7 hours over much of the gestation period and during the entire course of the pre-weaning period. The equivalent human exposure time would extend over several months.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- General lack of information pertaining to test animals, housing conditions and husbandry.
- Lack of detail with respect to culling of dams.
- Lack of detail with respect to pup selection.
- Presentation of results primarily in the form of graphs of limited resolution, with only limited numerical findings provided.
- Failure to include most conventional measures of toxicity as part of the study.
- Failure to perform replicate analyses of monoamine levels.
- Use of only two exposure concentrations.
- Lack of information pertaining to the number of samples analyzed and included in the statistical analyses.

Review – Confidence index ranking:

Moderate to low

Document Review

Author:	Skrajny, B., Reiffenstein, R.J., Sainsbury, R.S. and Roth, S.H.	**Internal ID:**	291
Title:	Effects of repeated exposures of hydrogen sulphide on rat hippocampal EEG		
Year:	1996		
Abstract:	*"Exposure to high levels of hydrogen sulphide (H_2S) in humans has been associated with a number of respiratory and neurological symptoms. Acute toxicity following exposure to high concentrations is well-documented, however, there is little scientific information concerning the effects of exposure to low concentrations. The effects of low levels of H_2S on electroencephalographic (EEG) activity in the hippocampus and neocortex were investigated on the freely moving rat (Sprague-Dawley). Hippocampal electrodes were implanted in the dentate gyrus (DG) and CA1 region). Activity was recorded for 10 min just prior to H_2S exposure in the presence of air (pre-exposure). Rats were exposed to H_2S (25, 50, 75 or 100 ppm) for 3h/day; data was collected during the final 10min of each exposure. The total power of hippocampal theta activity increased in a concentration-dependent manner in both DG and CA1; repeated exposures for 5 consecutive days resulted in a cumulative effect that required 2 weeks for complete recovery. The effects were found to be highly significant at all concentrations within subjects. Neocortical EEG and LIA (Large Amplitude Irregular Activity) were unaffected. The results demonstrate that repeated exposure to low levels of H_2S can produce cumulative changes in hippocampal function and suggest electivity of action of this toxicant."*		
Objective:	To examine the effects of inhalation of low levels of H_2S (25, 50, 75, 100ppm) on electroencephaographic (EEG) activity in the hippocampus and neocortex of the rat, with emphasis on investigating Large Amplitude Irregular Activity (LIA) (*e.g.*, grooming, shivering, immobility) and type 1 theta activity (*e.g.*, walking, running, rearing)..		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 25ppm; 50ppm; 75ppm; 100ppm (whole body)	Repeated/ 3 hours a day for five consecutive days	Rat	Sprague-Dawley	7-8 weeks of age	Male	5-6 rats per exposure concentration	Not specified.	NA	25ppm

Observations:

- Clinical signs: Limited to statement that *"eye irritation was never observed in the rats"*.

- 25ppm:
    - Total power of hippocampal theta activity:
        - Dentate gyrus (DG) region:
            - Day 1: ~ 5%increase
            - Day 2: ~ 13%increase
            - Day 3: ~ 19%increase
            - Day 4: ~ 22% increase
            - Day 5: ~ 26% increase
        - CA1 pyramidal region:
            - Day 1: ~ 2%increase
            - Day 2: ~ 5%increase

    - Day 3: ~ 15%increase
    - Day 4: ~ 14% increase
    - Day 5: ~ 27% increase*

- 50ppm:
  - Total power of hippocampal theta activity:
    - DG region:
      - Day 1: ~ 2%increase
      - Day 2: ~ 17% increase
      - Day 3: ~ 33% increase
      - Day 4: ~ 36% increase
      - Day 5: ~ 53% increase*
    - CA1 region:
      - Day 1: ~ 0%increase
      - Day 2: ~ 20% increase
      - Day 3: ~ 21% increase
      - Day 4: ~ 48% increase*
      - Day 5: ~ 66% increase*

- 75ppm:
  - Total power of hippocampal theta activity:
    - DG region:
      - Day 1: ~ 20%increase
      - Day 2: ~ 33% increase
      - Day 3: ~ 39% increase
      - Day 4: ~ 59% increase*
      - Day 5: ~ 78% increase*
    - CA1 region:
      - Day 1: ~ 18%increase
      - Day 2: ~ 30% increase
      - Day 3: ~ 37% increase
      - Day 4: ~ 72% increase*
      - Day 5: ~ 86% increase*

- 100ppm:
  - Amplitude of hippocampal theta activity:
    - CA1:
      - Peak frequency: No significant change
      - Percent of total power in 4-12 Hz: No significant change
    - DG: Increase
      - Peak frequency: No significant change
      - Percent of total power in 4-12 Hz: No significant change

- Behavior-EEG correlation: No change.
- Total power of hippocampal theta activity:
    - DG region:
        - Day 1: ~ 20% increase
        - Day 2: ~ 55% increase
        - Day 3: ~ 66% increase*
        - Day 4: ~ 92% increase*
        - Day 5: ~ 110% increase*
    - CA1 region:
        - Day 1: ~ 17% increase
        - Day 2: ~ 38% increase
        - Day 3: ~ 60% increase*
        - Day 4: ~ 82% increase*
        - Day 5: ~ 100% increase*
    - Power in both regions was still significantly increased one week after the last exposure, but returned to control levels at two weeks.
    - The "*second series of exposures to 100ppm H_2S produced almost identical effects on hippocampal theta as the first trial*" conducted a month prior.

- Effect of atropine sulphate on theta activity: No change.

*Statistically significant

Author's Conclusions:

- *"The most significant finding of this study is that repeated exposure to low concentrations of H_2S can produce a cumulative change in total power of hippocampal type 1 EEG activity, whereas LIA remained unchanged."*
- *"Exposure to H_2S altered theta activity in both CA1 and dentate gyrus regions. Both the power of hippocampal theta activity in the frequency window 4-12Hz, as well as noise levels (frequencies between 20-25 Hz) were increased, whereas the signal to noise ration remained unaltered during walking"*
- *"Since the effect on EEG activity by H_2S was not altered following administration of atropine sulphate, it is proposed that the observed changes were not due to the activation of the type 2 theta system. The lack of effect on LIA does suggest selectivity of action of H_2S on cell populations directly associated with voluntary movement."*
- *"The results demonstrate that repeated exposure to low levels of H_2S can produce cumulative changes in hippocampal function and suggest selectivity of action of this toxicant."*
- *"...the effects on hippocampal theta activity persisted for 1 week and required 2 weeks for complete recovery. This would suggest that the increase in total power of hippocampal theta activity is due to other mechanisms in addition to inhibition of cytochrome oxidase."*
- *"...it is possible that exposure to H_2S may cause precipitation of zinc as a sulphide salt. Depletion of the metal from hippocampal mossy fibers would result in alteration of neuronal activity observed as an increase in total power of hippocampal type 1 theta."*
- *"Further studies are necessary to confirm that H2S exerts differential effects on different brain regions via different mechanisms".*

Review – Assessment:

A. Test animals:	+/- The number of animals on test was somewhat limited (*i.e.*, n= 5-6 per exposure concentration). Guidelines for acute and subacute toxicity studies generally recommend use of 10 to 20 animals per exposure level. + Specie, stain, sex, age, weight variation and source of test animals were defined.

	- Weight variation of animals was slightly higher than that recommended by OECD guidelines. - Only males rats were exposed.
B. Exposure conditions:	+ The source of the H_2S gas was specified. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. As stated by the authors *"the effect of odorants on hippocampal theta activity must be considered."* - It is unclear whether animals were placed in the exposure chamber before or after the equilibrium period. - The total animal displacement in the exposure chamber was not reported. - Whether temperature, humidity or pressure was monitored in the exposure chamber was not recorded. + H_2S levels were monitored continuously, but actual concentration levels were not stated. + Airflow and oxygen concentration were monitored and complied with OECD guidelines.
C. Housing/Feeding:	+ The material from which housing cages were constructed was noted but the dimensions of these cages was not reported. - The location of the cages (*i.e.*, within chamber, in housing room, *etc.*) during non-exposure periods was not specified. - Whether animals were housed individually or in groups was not stated. + The source and type of feed was noted. - Details pertaining to the water supply were not provided. + Animals were fed *ad libitum*, except during exposure periods. This feeding schedule complies with OECD guidelines. + Bedding material was specified. +/- The environmental conditions under which the animals were housed (*e.g.*, temperature, humidity, etc) were not defined, except that a 12-hour photoperiod was maintained.
D. Equipment:	+ The material from which the exposure chambers were constructed (acrylic) and dimensions (33 L) were noted. - Whether the total animal volume exceeded 5% of the total chamber volume was not reported. + Exposure chamber was constructed such that continuous observation of the test animals was possible. +/- Gas concentration was monitored with a pre-calibrated digital GFG model GMA-100 monitoring system. - The manner by which airflow rate was monitored was not described. - If temperature and humidity were monitored, the equipment used to assess levels was not noted. +/- Theta activity was recorded with a polygraph tracer whose signals were amplified 10-fold and displayed on a digital oscilloscope.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. - No indication that rats were randomly assigned to exposure groups. +/- Two sets of controls were employed. First, EEG activity was monitored in rats exposed to air for 10 minutes prior to H_2S exposure to establish pre-exposure values. Second, rats assigned for exposure to 100 ppm of H_2S were first exposed to a nitrogen/air mixture for 3 hours per day for 5 days to establish baseline readings. It did not appear that a separate and distinct group of control rats was used. + This study was performed in accordance with the most recent *"Guide to the Care and Use of Experimental Animals"* prepared by The Canadian Council on Animal Care. + The behavior of the animals was continuously observed. - Unclear whether technicians and handlers were blinded to exposure conditions. - No indication that the study was performed under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- Conventional measures of toxicity (*e.g.*, clinical observations, body weight, gross pathology) were not performed. - Individual animal data were not supplied.

	- Table 1 values are not thoroughly identified, hampering interpretation. - Very little raw data were provided. Except for peak frequency and percent of total power, results were graphically displayed and resolution was generally lacking. +/- Reversibility of changes was assessed in five rats exposed to 100ppm. Similar analyses were not conducted for the other exposure groups.
G. Data analysis:	+ Statistical analyses were performed using GraphPad Instat. + One analysis *"involved a within subjects design utilizing a Friedman two-way analysis of variance"*. Data was also analyzed with *"a between group analysis of variance (ANOVA with Dunnett Multiple Comparison Test)."* - Confidence intervals were not provided.
H. Interpretations:	+/- Test animals served as their own controls, with baseline EEG readings taken before exposure to H_2S. A separate and distinct "sham" control group exposed to room air or a nitrogen/air mixture was not included.

Review - Summary:

Discussion of findings:

Sprague-Dawley rats exposed to low levels of H_2S for 3 hours/day for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in total hippocampal activity generated in the dentate gyrus (DG) and CA1 regions, as evidenced by changes in EEG readings compared to pre-exposure tracings. The changes in EEG readings reportedly extended to all treated groups, including the rats exposed to 25 ppm of H_2S. The mechanism by which H_2S induced the changes was unclear. However, it was determined that activation of type 2 theta activity (*i.e.*, arousal and sensory perception) was not involved as atropine sulphate administration did not alter the observed effects on the EEG. It would appear that the change was confined to alterations in type 1 theta activity, suggesting that the action of H_2S may be selective to cell populations directly associated with voluntary movement (*i.e.*, walking, grooming, rearing). Large Amplitude Irregular Activity (LIA) was unchanged by treatment, signifying that autonomic activity was not targeted. The observed changes in total hippocampal theta activity were reversed within 2 weeks following cessation of treatment. In contrast to the hippocampus, the EEG of the neocortex appeared unaffected by exposure.

Interpretation of the toxicological significance and clinical relevance of the findings should take into consideration the following:

- The study was not directed at the examination of the health effects *per se* caused by H_2S exposure, but rather was aimed at providing better understanding of the mechanism(s) surrounding the neurological effects associated with H_2S exposure, particularly effects claimed to be associated with low level exposures.
- The biological significance of the observed changes is unclear. Even the authors admit that further study is needed to "*confirm that H_2S exerts differential effects on different brain regions via different mechanisms*". Accordingly, the findings should be considered preliminary.
- Conventional measures of toxicity were not included as part of the overall study design. Thus, clinical and/or pathological correlates, if any, of the observed changes in EEG activity are unknown.
- The study used male rats only. This limits extrapolation of the findings to the human condition.

Confidence index:

The level of confidence that could be assigned to the study findings and conclusions was judged to be moderate. Interpretation of the toxicological significance and clinical relevance of the findings might have been improved through:

- Use of larger treatment groups. The use of only 5-6 rats per exposure concentration was somewhat limited and did not satisfy guideline recommendations.
- Use of both sexes of rats to ease extrapolation of the findings to the human condition.
- Addition of conventional measures of toxicity, including clinical observations and microscopic pathology of the affected tissues to determine if clinical

and/or histological correlates of the observed changes in EEG patterns exist.

- Use of a separate "sham" control group to permit better interpretation of the findings.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Struve, MF; Brisbois, JN; James, RA; Marshall, MW; and Dorman, DC	**Internal ID:**	296
Title:	Neurotoxicological effects associated with short-term exposure of Sprague-Dawley rats to hydrogen sulphide		
Year:	2001		
Abstract:	*"Although hydrogen sulfide (H_2S) is a known neurotoxic hazard, only a limited number of experimental animal studies have examined its neurochemical or behavioral effects. Our aim was to determine if short-term inhalation exposure of rats to H_2S would result in altered brain catecholamine levels or impaired learning and memory. Three groups of adult male CD rats were tested; two groups were exposed by nose-only inhalation (0, 30, 80, 200, or 400 ppm H_2S) and one group was exposed by whole-body inhalation (0, 10, 30 or 80 ppm H_2S) for 3 h per day for five consecutive days. The first group (n= 10 rats per concentration) was tested immediately following each daily nose-only H_2S exposure for spatial learning with a Morris water maze. Core body temperatures were also monitored in these animals during and after the last H_2S exposure. The second group of rats (n = 10 rats per concentration) was tested for spontaneous motor activity immediately following the fifth exposure. These rats were then euthanized and striatal, hippocampal, and hindbrain catecholamine levels determined. A third group of rats (n = 5-7 rats per concentration) was pretrained on a multiple fixed-interval (FI) schedule and exposed whole-body. Daily performance on the FI schedule was compared for the week pre-exposure, for the exposure week immediately following daily exposures, and for the week postexposure. We observed significant reductions in motor activity, water maze performance, and body temperature following exposure only to high concentrations (≥ 80 ppm) of H_2S. Exposure to H_2S did not affect regional brain catecholamine concentrations or performance on the FI schedule. Additional studies using other measures of behavior and longer-term exposure to H_2S may be required to more definitively address conditions under which H_2S exposure results in behavioral toxicity."*		
Objective:	To determine if short-term inhalation exposure of rats to hydrogen sulphide would result in behavioral toxicity and/or neurotoxicity, as evidenced by changes in brain catecholamine levels or impaired learning or memory. Both whole-body and nose only exposures were studied.		

Overall study design:

Exposure level (ppm)	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
(a) Whole-body: 0ppm; **10**ppm; **30**ppm; **80**ppm (b) Nose only: 0ppm; **30**ppm; **80**ppm; 200ppm; 400ppm	Repeated/ 3 hours a day for 5 consecutive days	Rat	Sprague-Dawley	(a) 10-14 weeks at time of exposure (b) 11-12 weeks at time of exposure	Male	(a) Varied depending on testing parameters.[1] (b) Varied depending on test parameters.[2]	Acclimated and presumed healthy	**30** ppm	**80** ppm (?)

1. Group sizes in Experiment (a) were as follows: 6 rats per concentration tested for spontaneous motor activity; 10 rats per concentration tested for spatial learning in a water maze; and, 5-7 rats per concentration tested for "FI" schedule performance

2. Group sizes in Experiment (b) were as follows: 10 rats per concentration tested for spontaneous motor activity; 10 rats per concentration tested for spatial learning in a water maze; 10 rats per concentration tested for rectal temperature (following water maze testing); and, 10 rats per concentration tested for brain catecholamine levels.

Observations:

- Learning and memory (water maze):
  - Whole body exposure (0, **10**, **30** or **80** ppm): No effect

- Nose-only exposure (400 ppm): Decreased performance, as evidenced by increased latency to find target "platform"*
  (200 ppm): Decreased performance
  (**30** or **80** ppm): No effect

- Motor activity:
  - Whole body exposure (0, **10**, **30** or **80**ppm): No significant effects.
  - Nose-only exposure (**80**, 200, and 400ppm): Decrease in ambulatory and total movements*

- Fixed interval operant behavior: No significant changes in fixed operant behavior following whole-body exposures (0, **10**, **30** or **80** ppm) as measured by performance on an "*F1 schedule*" involving conditioned reward responses following fixed cues.

- Rectal body temperature:
  - Nose only exposure (200 and 400 ppm): Decreased*

- Regional brain catecholamine levels: No significant changes in brain catecholamine levels regardless of exposure concentration (0, **30**, **80**, 200, or 400 ppm). Catecholamines monitored included striatal, hippocampal and hindbrain dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid.

- One animal exposed to 400 ppm died on test. All other animals survived.

*Statistically significant

Author's Conclusions:

- *"We observed significant reductions in motor activity, water maze performance, and body temperature following exposure only to high concentrations (greater than or equal to 80 ppm) of H_2S. Exposure to H_2S did not affect regional brain catecholamine concentrations or performance on the F1 schedule".*
- *"The results of our research suggest that cognitive dysfunction is not anticipated to occur following short-term, repeated H_2S exposures to concentrations equivalent to current occupational exposure limits (10ppm)".*
- *"Reassuringly, our data suggest that acute human exposures to H_2S at levels associated with odor annoyance (i.e., multiples of the olfactory threshold) are unlikely to result in neurobehavioral toxicity. Additional studies using more prolonged exposure durations and additional measures of behavior are required to more definitively address whether low-level H_2S exposure is associated with neurotoxicity".*

Review – Assessment:

A. Test animals:	+ Number of rats per exposure concentration satisfies guideline recommendations. + The principle characteristics of test animals (*e.g.*, specie, age, sex, weight variation) were adequately reported; however, information pertaining pre-test health status was not provided. - Study limited to use of male rats only. + Age at initiation of exposure (10-14 weeks) was consistent with guideline recommendations. + Source and age of test animals was described.
B. Exposure conditions:	+ Source of H_2S gas was reported. + Graded exposure concentrations were studied, together with a concurrent control group for both exposure "scenarios" (*i.e.*, whole-body and nose only). + Chamber atmospheres were equilibrated prior to placement of animals inside the chambers.

	+ Target exposure concentrations were analytically confirmed. Measured concentrations were consistently within 5% of target. Concentrations were determined at least 6 times during each 3-hour exposure. + Airflow (200 to 250 L/min), temperature (20.3 to 21.7^{0}C) and humidity (31 to52%) within chambers were monitored.
C. Housing/Feeding:	+ Adequate descriptions of housing conditions and feeding schedule. +/- Animals housed individually or in pairs in polycarbonate cages with cellulose fiber bedding. + Animals fed commercial rodent chow on an *ad libitum* basis. + Temperature (18.5-21.5^{0}C), relative humidity (40-70%) and photoperiod (12-h light-dark cycle) of animal room were consistent with guideline recommendations. +/- Water supply consisted of deionized, filtered tap water, provided on an *ad libitum* basis. + Bedding type was specified.
D. Equipment:	+ Adequate description of exposure equipment, including details of chambers used for whole-body and nose only exposures. Additional details available in companion paper (Dorman *et al.*, 2000 – Study ID 149).
E. Procedural:	+ Test animals acclimated for two weeks prior to exposure, consistent with guideline recommendations. + Rats were randomly assigned to exposure groups. + Control exposures were performed, consistent with guideline recommendations. + Graded exposure concentrations were tested, consistent with guideline recommendations. - Nose-only exposures were performed in series, thereby introducing potential confounding influences. + Exposure schedule was 3 hours per day for 5 days, consistent with definition of short-term exposure. + Behavioral endpoints were analyzed within 20 min of the end of exposure. + The Morris water maze test was completed five times daily by each rat to reduce the impact of experimental error on results. + Animals were euthanized by decapitation. + Conducted under the National Research Council's federal guidelines for the use and care of laboratory animals. + Study design was approved by the CIIT Institutional Animal Care and Use Committee. - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	+/- Data collection was targeted at endpoints for assessment of neurotoxicity, including indicators of memory and learning. Tests included water maze performance, fixed interval operant behavior and spontaneous motor activity. - Conventional measures of toxicity were not performed (*i.e.*, clinical observations, body weights, feed consumption, clinical pathology, organ weights, gross pathology and/or histopathology). - Individual animal data were not supplied. - Many of the findings were reported in the form of figures (*i.e.*, graphs), some of limited resolution. Presentation of numerical data was limited to brain catecholamine levels.
G. Data analysis:	+/- Statistical methods were adequately described. Separate and distinct analyses were performed for each of the various measures of neurotoxicity examined.
H. Interpretation:	- Findings apply only to male rats. Females were not included in the study. - Some inconsistency in motor activity results at 80 ppm depending on mode of exposure. Whole-body exposure at 80 ppm showed no change in motor activity compared to control exposure, but motor activity was decreased following nose only exposure to 80 ppm. +/- Interpretation necessarily limited to effects of H_2S on neurological function since conventional toxicity endpoints were not included as part of the study.

Review - Summary:

Discussion of findings:

Repeated, short-term inhalation exposure of male rats to concentrations of 0, **10**, **30**, **80**, 200 or 400 ppm H_2S for up to 5 days produced dose-related changes in certain measures of neurotoxicity, including learning and memory. Changes were confined to the higher dose levels (*i.e.*, greater than or equal to **80** ppm) and were dependent, in part, on the mode of exposure (*i.e.*, whole-body versus nose only). The effects on learning and memory were not consistent across all test measures employed, with the high-dose rats (400 ppm) showing reduced water maze performance, but no changes in fixed interval operant behavior. At the higher exposure concentrations, a reduction in spontaneous motor activity also was demonstrated, but the response at **80** ppm was inconsistent and varied depending upon the mode of exposure. The motor activity of rats receiving whole-body exposures to **80** ppm was unchanged, while animals that received nose only exposures showed reduced activity. At exposure concentrations greater than 200 ppm, rectal temperature was decreased. No changes in brain catecholamine levels were demonstrated at any exposure concentration. Exposure concentrations of **10** and **30** ppm were without effect on any of the parameters monitored. A single rat exposed to 400 ppm died. All other animals survived the full schedule of exposures (*i.e.*, 3 hours per day for 5 days). The NOEL for the study was judged to be **30** ppm, with a LOEL of **80** ppm based on an apparent reduction in spontaneous motor activity.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The primary objective of the study was not to investigate the health effects *per se* of H_2S, but rather the aim was to specifically examine the effects of exposure on neurochemistry and neurobehavioral function. As a result, the study was limited in scope and did not include most routine measures of toxicity (*e.g.*, clinical observations, body weights, clinical pathology, gross pathology).
- Changes were confined to the high exposure concentrations only. The rats exposed to **10** ppm or **30** ppm were unaffected by treatment. The only reported change observed at **80** ppm was a reduction in spontaneous motor activity following nose-only exposure. The change was significant at only 2 of 10 testing intervals. A similar response was not evident following whole-body exposure. In addition, the rats exposed to **80** ppm showed no deficits in learning and memory, as evidenced by normal performance in a water maze, and no changes in fixed interval operant behavior, as evidenced by normal performance on the "F1" schedule. Given the inconsistency of the response and the lack of progression of the change over the course of treatment, combined with the lack of response for all other measures of neurobehavioral function, the change in spontaneous motor activity following exposure to **80** ppm may be equivocal.
- Testing was completed using male rats only. Extrapolation of the findings to the human condition would have been improved through testing with both sexes.
- The clinical relevance of the observed changes, including the reduction in spontaneous motor activity following nose-only exposure, is unknown. It is especially difficult to assign clinical significance to the modest and inconsistent changes in the ambulatory movements of rats observed following exposure to **80** ppm of H_2S.

Confidence index:

Study design, conduct and reporting were judged to be adequate for the purposes of the investigation. Any deficiencies noted were generally minor in nature. Greater confidence in the study findings and conclusions could have been achieved by:

- Inclusion of both female and male rats.
- Assessment of conventional measures of toxicity.
- More complete reporting of results, with greater reliance on numerical data.

Review – Confidence index ranking:

Moderate to high

Document Review

Author:	Weedon, FR; Hartzeli, A; Setterstrom, C	**Internal ID:**	316
Title:	Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. V. Animals		
Year:	1940		
Abstract:	*Not available*		
Objective:	To examine the toxicity of a series of industrial gases to animals following exposure under controlled conditions involving continuous flow. (Note: The present review is concerned with the portion of the study directed at the examination of the toxicity of hydrogen sulphide to rats and mice. Those portions of the study aimed at the examination of the toxicity of ammonia, chlorine, hydrogen cyanide and sulphur dioxide were not subject to detailed review, apart from information common to all of the gases. A separate portion of the study directed at the examination of the effect of H_2S on houseflies also was not subject to detailed review).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
16ppm; **63ppm;** 250ppm; 1000 ppm	Single/ 16 hr. Animals which survived exposure were held up to 5 months for observation.	Rat, mouse	Not specified	Not specified. The animals were described as *"young, vigorous, and mature"*.	Both	Eight rats and four mice per exposure concentration tested. Number of animals was not differentiated by sex. Control animals also were included, but numbers were not specified.	Not specified	16 ppm	63 ppm

Observations:

- Clinical observations:
  - Dose-related symptoms, ranging from slight restlessness to distinct hyperactivity, followed by lethargy, loss of muscular coordination, staggering, coma and/or death. At the higher concentrations (> 250 ppm), preening of the facial area, with or without marked lacrimation was evident, suggestive of irritation of the eyes and nose.
  - The mice were more markedly affected than the rats.

- Mortality:
  - 1000ppm: All rats and mice died
  - 250ppm: Three of 8 rats died; all mice died
  - **63**ppm: One of 8 rats died; all mice died
  - **16**ppm: All rats and mice survived.

- Time to 50% mortality (LT_{50}):
  - 1000ppm: 14 to 18 minutes
  - 250ppm: 7 to greater than 16 hours

  - 63ppm: 13 to greater than 16 hours
  - 16ppm: No deaths.

- Time until deaths recorded:
  - 1000ppm: 20 to 40 minutes
  - 250ppm: 7 to 23 hours
  - 63ppm: 1 to 40 hours
  - 16ppm: No deaths.

- Necropsy (animals that died during exposure):
  - Light to marked congestion of the brain, liver and/or kidneys.
  - Dilatation of the heart.
  - Distention of the stomach, gall bladder and/or intestines.
  - Minor to massive hemorrhagic infiltration of the lungs, and/or pale discoloration of the liver, kidneys and/or adrenals.
  - Findings were similar in both rats and mice.

- Necropsy (animals that survived exposure and were maintained for 5 months)
  - No remarkable findings.

*Statistically significant

Author's Conclusions:

- *"The rats were not markedly affected during the first hour of exposure to H_2S* ***63*** *ppm but continued to eat and drink undisturbed. Even after 16 hours' exposure the seven surviving rats appeared to be in fair condition, although lethargic and breathing heavily. The mice showed similar but more marked signs than the rats".*
- *"On initial exposure to* ***16*** *ppm the animals showed some slight restlessness but quickly became habituated, and showed no other abnormal symptoms at this concentration".*
- *"At high concentrations of H_2S there was violent activity and loss of muscular coordination...; at progressively lower concentrations there was progressively less activity. In addition in the lower concentrations there was evidence of irritation of the eyes and nose and of pulmonary edema increasing with time of exposure".*

Review – Assessment:

A. Test animals:	+/- Number of test animals limited to 8 rats and 4 mice (both sexes) for each dose level, as opposed to recommended 5 animals per sex per dose level. Number of male animals versus number of females tested was not specified. However, the number of animals was judged to be sufficient for the purposes of the study. - No information was supplied concerning the strain, source or health status of the test animals prior to study initiation. +/- Age of test animals was not specified, rather they were described as *"young, vigorous and mature"*. - Body weights of test animals at initiation were not provided. - Details concerning control animals were largely lacking (*i.e.*, described only as being of same age as test animals, numbers were not supplied).
B. Exposure conditions:	+/- Animals were exposed to **16**, **63**, 250 or 1000 ppm H_2S under *"continuous flow"* conditions.

	- No record that reported test concentrations of the gas were analytically confirmed. +/- Duration of exposure varied up to 16 hours or until time of death. +/- Some evidence that temperature and humidity within the exposure chamber were monitored, but degree of control could not be discerned.
C. Housing/Feeding:	+/- Basic details respecting housing during treatment were provided. Animals were housed in wire cages during exposure (but information was not supplied as to whether or not the animals were caged singly or gang-caged). Temperature (73 ^{0}F) and humidity (75%) at time of treatment were recorded (albeit humidity level was higher than generally recommended). - Details concerning bedding materials were not supplied. +/- Details concerning feed supply were provided, including proximate analysis; however no information was supplied respecting contaminant analysis. Feed was provided *ad libitum*. - Details concerning water supply were generally lacking. Water was provided on demand. +/- Limited reference to need to control photoperiod (*i.e.*, measures were taken to minimize light fluctuations).
D. Equipment:	+/- Details concerning the gas delivery system used in the study were provided in a series of companion papers (Setterstrom and Zimmerman, 1938; McCallan and Setterstrom, 1940). + Attention was given to controlling gas flow, maintaining exposure concentrations in the chamber, regulating temperature and humidity, and analytically confirming exposure concentrations. - Equipment was necessarily 'crude' by present day standards, with little automation, and reliance on stopwatches and "*warning bells*". Accuracy and precision of calibration methods and analytical techniques was judged to be questionable. - Details concerning the equipment were largely for studies involving exposure to SO_2 only. No information was supplied concerning equipment modifications and changes in calibration methods for exposures with H_2S. - Details with respect to the analytical methodology used to measure the concentrations of H_2S were lacking. The available information indicated only that the chamber atmosphere was monitored continuously with an "*autometer*", and that the concentration of H_2S was determined by measuring the conductivity of an "*absorbent*" generated by passing the gas through a lead acetate solution.
E. Procedural:	- No indication that test animals were quarantined or acclimatized prior to treatment. - No details supplied concerning randomization of test animals and assignment to test groups. +/- Reference to use of control animals as part of study, but no details supplied. + Evidence that animals that died on test were either subject to necropsy immediately or stored under refrigerated conditions until necropsy could be scheduled in order to avoid tissue autolysis. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	+ Clinical observations were performed during treatment, and included time of onset of symptoms, duration of symptoms and severity of responses. + Mortality data were provided, including time to death. - Body weight data were not collected. + Necropsies were performed on all animals, and included visual observation of major organ systems. - Organ weight data were not recorded as part of necropsy procedures. + Evidence that major viscera were preserved for possible future histological examination. - Individual animal data were not supplied.
G. Data analysis:	+/- Data analysis consisted of construction of time-mortality curves on logarithmic-probability coordinates. - No further analyses of the study findings were completed.
H. Interpretation:	- The accuracy and precision of the analytical methods used to measure the exposure concentrations of H_2S are questionable.

	+/- The study was performed using rats and mice, thereby requiring extrapolation of the findings to the human condition.

Review - Summary:

Discussion of findings:

Rats and mice exposed to graded concentrations of H_2S for up to 16 hours under controlled conditions showed dose-dependent signs of intoxication, ranging from mild restlessness to hyperactivity, coma and death. Mice were more markedly affected than rats. The lowest concentration tested (**16** ppm) produced only mild, transient restlessness during the initial stages of exposure, with no other evidence of intoxication despite continued exposure for 16 hours. Necropsy findings at this concentration were uniformly non-remarkable. At **63** ppm, frank evidence of intoxication was presented, especially among the test mice, with deaths recorded as early as within one hour of exposure and 100% of the mice dying within 40 hours. The test rats appeared more resistant, with only one of 8 animals dying on test. Necropsy findings from these animals showed hemorrhagic infiltration of the lungs and congestion and/or discoloration of the brain, liver and/or kidneys consistent with intoxication. Clinical signs and necropsy findings were more remarkable among the test animals exposed to the highest concentrations (250 and 1000 ppm).

Interpretation of the significance of the findings should take into consideration the following:

- The time to mortality curves for both the rats and mice showed very steep responses, suggesting that concentration is the major determinant of toxicity for H_2S. Once the threshold dose for toxicity was exceeded, the animals quickly succumbed.
- The mice were very markedly affected, with all animals dying within 40 hours of exposure to H_2S. Rats were less severely affected, indicating distinct species differences in response.
- The study is dated and was performed long before the development of testing guidelines and the introduction of Good Laboratory Practice (GLP) requirements. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively “crude” instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested.
- Much of the description of exposure conditions and the gas delivery system related to sulfur dioxide. There was very little discussion surrounding modifications, if any, that were performed to allow for the controlled delivery and analysis of H_2S. The lack of information acts to erode confidence in the study findings.

Confidence index:

The confidence index of the study was judged to be moderate-to-low. The overall study design and conduct were adequate for the purposes of the investigation. Reporting was lacking in several respects. Much of the description of the gas delivery system and the analytical methods used to measure the concentration of the gas in the exposure chamber was not specific to H_2S, but rather related to SO_2. Accordingly, some uncertainty surrounds the actual concentrations of H_2S to which the test animals were exposed. Confidence in the study findings could have been improved by:

- Better description of exposure conditions and the gas delivery system, as specifically related to the exposures involving H_2S.
- Provision of data for the control group of animals.

Review – Confidence index ranking:

Moderate to low

Document Review

Author:	Lodgepole Blowout inquiry panel	**Internal ID:**	327
Title:	Lodgepole blowout report. Causes, effects, actions.		
Year:	1984		
Abstract:	*Not available*		
Objective:	A detailed analysis of the Lodgepole blowout and its effect on property and human/ wildlife health.		

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Less than 15 ppm.	*"During the 26 days…H_2S concentrations at, or near, residences exceeded 10ppm for about 31 hours in total, and 5 ppm for 61 hours."* *"On six occasion, H_2S concentrations near residences were in excess of 15ppm and residents temporarily left the area."* *"…at Lodgepole, the concentrations were less than 1ppm for 87 per cent of the recorded hours, while at Cynthia and Drayton Valley they were less than 0.1ppm for 93 per cent of the time."*	Not specified.	Not specified.	30	Not specified.	NA	NA

Observations:

- 30 residents testified at inquiry:
  - *"…symptoms had an onset coincidental with the blowout. These included headache, eye irritation, upper respiratory tract symptoms such as sore throat, nasal irritation, and nosebleeds in children, lower respiratory tract symptoms including pain on deep inhalation and some shortness of breath."*
  - *"Gastrointestinal problems such as loss of appetite, nausea, and diarrhea were also described."*
  - *"These complaints were said to improve within 24 hours in people who left the area and recurred within 24 hours when the same individuals returned"*
  - *"When the well was on fire, some people recalled improvement while others reported that they suffered persistent symptoms."*
  - *"Complaints suggestive of central nervous dysfunction were infrequent"*
  - *"…individuals denied symptoms such as chills, generalized muscle aching, or some of the usual systemic complaints of an acute infectious process"*
  - *"Two…witnesses with previously diagnosed emphysema, said that they were considerably affected and the asthmatic husband of a third was described as incapacitated."*

*Statistically significant

Author's Conclusions:

- *"Many individuals living in the Lodgepole region suffered from headache, respiratory, and gastrointestinal symptoms during the blowout. These people were exposed to H_2S during this time and it is probable that their symptoms were caused by this exposure."*
- *"...interpretation of these symptoms suggests that some could be related to irritation of exposed mucous membranes or gastrointestinal dysfunction, while other symptoms such as headache cannot be identified with dysfunction of one organ system."*
- *"Various interpretations were placed on these submissions. Some participants at the inquiry contended that the problems related to a "flu epidemic", others to psychologically inducted reactions. Others claimed that the noxious gas was the cause."*
- *"Because of the lack of data, it was very difficult to assess the effect of the blowout on groups which might be expected to be at special risk..."*
- *"...lack of scientific data available for analysis. Considering the large amount of circumstantial evidence, however, the Panel is satisfied that the well emissions did lead to at least short-tem health effects..."*
- *"The anecdotal evidence also strongly suggests that some people, especially those having respiratory problems, are much more likely to suffer unfavorable effect from H_2S gas."*
- *"With respect to emissions of other components from the blowout well the Panel is not able to make any assessment because there is no data available."*
- *"The condition of patients with chronic respiratory disease in Edmonton and areas remote from the blowout site who were exposed to low concentrations of pollutants may have been temporarily aggravated, but insufficient data was available to evaluate this possibility."*

Review – Assessment:

A. Subjects:	+/- The number of subjects was deemed to be adequate, but was somewhat limited. - The age, sex, health and patient history of the witnesses was not provided. +/- It was stated that an *"attempt was made by the Panel to determine how representative this small group would be of the entire community"* but the outcome of this investigation was not provided. - Subjects were members of the Pembina Area Sour Gas Exposures Committee (PASGEC).
B. Exposure conditions:	- Both the precise concentration and duration of exposure for the individual witnesses were not indicated. + It might be possible to estimate the exposure levels since the report includes the location of the residences of the witnesses. - *"The panel...does not believe that the hourly average contours calculated on the basis of mobile monitoring data are a valid representation of the actual concentrations. In its view, there is insufficient data to derive meaningful average hourly concentrations"* - The identity and concentration of other released substances were not reported.
C. Equipment:	+ Stationary air monitors were installed in the nearby communities and there were five mobile monitors used to check rural areas. +/- *"criticisms related to whether there were enough monitoring units to satisfactorily cover the entire area potentially affected"* - Monitors had a limited concentration range for which they could accurately assess H_2S levels. The five mobile monitors had limits of 10, 10, 5, 1, and 30ppm H_2S, whereas, stationary units had recording capacities up to 3.27, 5, 5, and 1ppm. - Monitoring at higher levels was completed using relatively crude assessment tools (*e.g.*, Drager units, and Dictaphone instruments)

D. Procedural:	- The only description of health effects provided was the testimony of the 30 residents. - The symptoms were not confirmed by medical practitioner(s). - To determine how representative the witnesses were of the general population, the panel reviewed data from a previous report which employed a questionnaire to compare the frequency of symptoms experienced before, during and after the blowout. In the earlier report, symptom frequency appeared to be elevated, but the increase was not statistically confirmed. - The frequency of symptoms in a control community was not assessed. - Blinding was not possible. + The testimony was given within 2 years of the blowout. - The case was *"widely publicized at pre-inquiry meetings"*, hence bias is a concern.
E. Data collection:	- Information pertaining to the severity, onset and dose-response relationship was not provided for any symptom. - Medical records were not provided to verify claims. - The nature and severity of symptoms was not graded against an explicitly defined scale. - No raw health effects data were provided to permit the reader to arrive at his/her own conclusions. - Individual data were not provided for each subject. - Exposure estimates were not correlated with individual witness testimony.
F. Data analysis:	- No statistical analyses were conducted.
G. Interpretations:	+/- The role of additional chemicals was not investigated. However, authors did explore acute infectious diseases and stress induced psychological effects as possible explanations for the reported health outcomes.

Review - Summary:

Discussion of findings:

The Lodgepole well remained unignited for 26 of the 67 days that the well was out of control. During this time, the concentration of H_2S near residences exceeded 10ppm for 31 hours and 5ppm for a period of 61 hours. Residents within a 50 km radius complained of headache, eye irritation and upper/lower respiratory tract symptoms coincidental with the blowout. Evacuated individuals reported that symptoms cleared within 24 hours, but returned upon relocating to the blowout area. Those with chronic respiratory problems appeared to suffer more acutely from exposure than 'healthy' individuals.

Interpretation of the significance of the study findings should take into consideration the following:

- The purpose of this report was to provide a detailed analysis of the Lodgepole blowout, to describe its impact on the environment and human health and to provide recommendations for the industry. Consequently, this document presents only a cursory review of the human health issue. It is based upon the testimony of only 30 residents. As stated by the authors, there is a "*...lack of scientific data available for analysis*".
- It is not known to what degree the witnesses were representative of the general population within the communities surrounding the well-site.
- Evidence of hypersensitivity was based on testimony of three individuals. As stated by the authors' *"Because of the lack of data, it was very difficult to assess the effect of the blowout on groups which might be expected to be at special risk"*.
- No individual subject data were provided.
- *"The Panel was not presented with any medical studies"*.
- Exposure monitoring was highly criticized in the inquiry. In fact, the Panel concluded that *"there is insufficient data to derive meaningful average hourly concentrations"*.
- The role of other chemical agents in inducing observed symptoms was not explored. It is stated that *"With respect to emissions of other components from the blowout well the Panel is not able to make any assessment because there is no data available."*

Confidence index:

The level of confidence that could be assigned to reported findings and conclusions was deemed to be low. Several weaknesses in the investigation and reporting were evident. The weaknesses included:

- Lack of reliable exposure estimates.
- Possible subject bias. Bias may have been introduced through the manner in which witnesses were recruited.
- An absence of any medical data to confirm the reported symptoms.
- Failure to account for chemical exposures apart from H_2S that may have contributed to the observed symptoms.

Review – Confidence index ranking:

Low

Document Review

Author:	Nicholson, RA; Roth, SH; Zhang, A; Zheng, J; Brookes, J; Skrajny, B; Bennington, R	**Internal ID:**	398
Title:	Inhibition of respiratory and bioenergetic mechanisms by hydrogen sulfide in mammalian brain.		
Year:	1998		
Abstract:	*"The biochemical effects of hydrogen sulfide were investigated by treating enzyme homogenates and synaptosomes prepared from mammalian brain with sodium sulfide. Brain cytochrome c oxidase activity was highly sensitive to inhibition by sodium sulfide, as demonstrated by an IC50 of 0.13 µ. Sodium sulfide was also found to inhibit carbonic anhydrase activity in cerebellum, frontal cortex, and hippocampus. Synaptosomal oxygen consumption was significantly reduced as the concentration of sodium sulfide was increased from 20 to 100 µM; this was accompanied by a concentration-dependent depolarization of the synaptosomal mitochondrial membrane in situ and a reduction in synaptosomal ATP concentration. In other experiments using synaptosomes, sodium sulfide caused a significant calcium-independent increase in the extracellular accumulation of L-glutamate, inhibited Na^+-dependent uptake of (^3H)glutamate, but was unable to influence intrasynaptosomal free ionic Ca^{2+}. Parallel studies conducted in vivo showed that rats exposed over a 5-d period to hydrogen sulfide (100 ppm for 3 h/d) had significantly higher concentrations of L-glutamate in the hippocampus compared to control animals. In summary, our results indicate that sulfide causes extensive disruption to respiratory and related mitochondrial functions in mammalian brain in vitro. The reduced capacity of nerve endings to take up L-glutamate may contribute to the raised L-glutamate levels observed in vivo."*		
Objective:	A three-part experiment to determine the effects of H_2S on brain function at the biochemical level. Part (a) determined the susceptibility of brain cytochrome *c* oxidase and carbonic anhydrase to sulfide inhibition *in vitro*; Part (b) examined the actions of sulfides on synaptosomal function; Part (c) examined the potential changes in hippocampal L-glutamate concentrations following H_2S exposure *in vivo*. Only Part (c) was subject to review.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
0 ppm; 100ppm (whole body)	Repeated 3 hours a day for 5 consecutive days	Rat	Sprague-Dawley	Not specified.	Male	24. (12 treated; 12 control)	Not specified.	NA	100ppm

Observations:

- Glutamate levels in hippocampus:
  - 100ppm: 24% increase* relative to control value

*Statistically significant

Author's Conclusions:

- *"Exposure of rats to gaseous hydrogen sulfide (100ppm for 3h/d for 5 d) caused a significant (24%) increase in glutamate levels in the hippocampus."*
- *"...results indicate that in addition to bioenergetic and respiratory effects, increased stimulation of glutamate receptors may contribute to the neurotoxic mechanisms of action of hydrogen sulfide in mammalian brain."*

Review – Assessment:

A. Test animals:	+ Adequate number of test animals were used (*i.e.*, n=12 per group) + -Most of the primary characteristics of the test animals (*e.g.*, specie, strain, sex, weight variation and source) were reported but

	the age and pre-test health status were not specified. +/- Weight variation of test animals (*i.e.*, 25%) was slightly higher than that recommended by OECD guidelines. - Only males rats were used. - Animals were handled in accordance with the Canadian Council on Animal Care guidelines
B. Exposure conditions:	+ The source of the H_2S gas was reported. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It is unclear whether animals were placed in the exposure chamber before or after the equilibration period. - The total animal displacement in the chambers was not reported. - Whether H_2S concentration, oxygen content, temperature, pressure, humidity and photoperiod were monitored throughout exposure was not reported.
C. Housing/Feeding:	+ Details concerning animal housing (*i.e.*, temperature, humidity and photoperiod) were provided, and were within guideline recommendations. +/- Animals were gang- caged, but the number of animals per cage and how caging groups were allocated was not specified. - Information pertaining to animal caging *(i.e.*, material and dimensions) was not provided. - Bedding material was not specified. - The type and source of feed supplied was not reported. +/- Animals were reportedly *"given continuous access to food and water"*. Whether this access continued during exposure was unclear. - Details concerning the water supply were lacking.
D. Equipment:	+/- Descriptions of the exposure chamber and gas delivery system were minimal as they were described previously in Skrajny *et al.*, 1996 (Study ID-291)
E. Procedural:	- No acclimation period was specified. - No description of pre-test conditions was provided. - Absence of data pertaining to the randomization of test animals and assignment to test groups. + A control group was included and the control animals were exposed simultaneously. - Unclear whether technicians and handlers were blinded to exposure conditions. - Conventional measures of toxicity (*i.e.*, clinical observations, body weights, clinical pathology, gross pathology) were not monitored as part of the study. - A single exposure concentration only was tested, thereby precluding any determination of dose-responsiveness. Testing guidelines generally the use of three treatment levels and a corresponding control group as part of a subacute toxicity study.. +/- Animals were anesthetized with halothane and decapitated, but it was not indicated whether animals were randomly sacrificed. - It would appear that only single analyses of brain glutamate levels were performed (i.e., analyses were not performed in replicate). + All biochemical assay methods were referenced. - Whether biochemical analyses of samples were conducted in random order was not indicated. - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions..
F. Data collection:	- Individual data were not provided for each test animal, thereby limiting the independent assessment of findings. + All observations were objective.
G. Data analysis:	+ Statistical analysis included both an analysis of variance (ANOVA) and Student's t test. - Confidence intervals were not provided.

H. Interpretations:	- Testing was conducted on male rats only. - Dose-responsiveness of reported changes in brain glutamate levels could not be determined only to the use of a single exposure concentration only. - The toxicological and clinical relevance of the observed change in brain glutamate level is not known.

Review - Summary:

Discussion of findings:

Whole-body exposure of male rats to 100 ppm of H_2S for three hours per day for 5 days under controlled conditions resulted in increased levels of L-glutamate in the hippocampal region of the brain compared to control rats. It was postulated that the change in glutamate levels might contribute to the neurotoxic mechanisms of action of H_2S in the mammalian brain.

Interpretation of the toxicological significance and clinical relevance of the observed changes should take into consideration the following:

- The primary objective of the study was not to examine the health effects *per se* of H_2S, but rather to elucidate the possible mechanisms of action of H_2S within the mammalian brain.
- The toxicological significance and clinical relevance of the increased levels of L-glutamate is not known. Although the authors claimed that because of its role as a neurotransmitter, alterations in L-glutamate levels might contribute to the neurotoxic mechanisms of action of H_2S in the brain, no clinical or pathological correlates of the observed changes were reported owing to limitations in study design. Interpretation of the significance of the changes in brain glutamate levels might have been improved by the addition of routine measures of toxicity to the overall study design (*i.e.*, the study lacked any clinical observations and pathological examination of tissues).
- The dose-responsiveness of the observed changes in glutamate levels could not be determined owing to the use of a single exposure concentration only. Interpretation of the significance of the findings might have been improved through the use of graded exposure levels, consistent with guideline recommendations for subacute toxicity studies.
- The study used male rats only. Extrapolation of the findings to the human condition would have been improved by extending the testing to include both sexes.
- The findings should be considered preliminary in nature only. As the authors admit: *"…future experimentation on the ability of hydrogen sulfide to interfere with the metabolism is warranted"*.

Confidence index:

Overall confidence in the study findings was judged to be moderate. Several weaknesses in study design, conduct and/or reporting detracted from the level of confidence. The weaknesses included:

- Use of a single exposure concentration only. Testing guidelines for subacute toxicity studies generally recommend the use of three exposure levels and a concurrent control group.
- Failure to measure any parameters other than L-glutamate levels in the brain. Conventional measures of toxicity, such as clinical observations, body weights, gross pathology, organ weights and histopathology, were ignored.
- Use of male rats only. Testing guidelines generally recommend the use of both sexes.

Review – Confidence index ranking:

Moderate

Document Review

Author:	Hannah, R.S.; Hayden, L.J.; Roth, S.H.	**Internal ID:**	402
Title:	A relationship between hydrogen sulfide exposure and taurine levels in maternal rats.		
Year:	1990		
Abstract:	*"Hydrogen sulfide is an environmental and industrial pollutant. The effect of prolonged exposure to low concentrations is not well established, however, it has been described as a form of chronic poisoning [1].* *We have previously demonstrated that exposure to low concentrations of hydrogen sulfide (H_2S) produces a significant increase in the level of taurine in the developing rat CNS which returns to control values at the same approximate time as the establishment of the blood-brain barrier to taurine [2]. The purpose of this study was to establish if this observed increase could be related to increased maternal plasma levels of taurine."*		
Objective:	To investigate the effect of exposure to H_2S on maternal plasma levels of taurine in rats. (Plasma taurine levels were monitored at parturition and at Day 21 post-partum).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 ppm; 50ppm	Repeated/ 7 hours a day from day 6 postcoitus until day 21 postnatal.	Rat	Sprague-Dawley	Not specified	Female	Not specified.	Timed-pregnant	NA	50ppm

Observations:

- Maternal blood plasma levels of taurine:
  - At parturition: ~ 35% higher in treated rats
  - At Day 21 post-partum: ~34% higher in treated rats

*Statistically significant

Author's Conclusions:

- *"Maternal blood plasma taurine levels in the treated dams were found to be significantly increased over control levels at both sample times…the approximate increase was 30%."*
- *"…the mechanism which produced the increased taurine levels was initiated prior to partuition resulting in the stable 30% increase between birth and weaning."*
- *"Since taurine is rapidly transferred from the dam to the pups both transplacentely and via the milk, it is highly probable that the pup blood plasma levels of taurine were still elevated on day 21 pn after CNS levels had declined to normal values."*
- *"The excess taurine present during this phase of growth would have the potential to produce neuronal abnormalities."*

Review – Assessment:

A. Test animals:	- The number of animals used in the study was not stated. +/- Some of the principle characteristics of test animals (e.g. specie, strain, sex and pre-test health) were reported but the age, weight variation and source of animals were not provided.
B. Exposure conditions:	- The source of H_2S gas was not reported. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially affect the duration of exposure. + The exposure device was adequately described. + The total animal displacement in exposure chambers did not exceed 5% of the total volume of the chamber, consistent with guideline recommendations.. + The exposure chamber was designed to permit continuous observation of animals. +/- Exposure concentrations, airflow (20 changes per hour), temperature, pressure, humidity and oxygen content within the exposure chamber were monitored throughout exposure; however, actual conditions were only stated for selected parameters. +/- Actual concentrations of H_2S were reportedly measured at the animal level, but the difference between measured and target levels was not stated.
C. Housing/Feeding:	- Details concerning housing conditions were lacking. It was unclear as to whether the dams were housed in the exposure chamber continuously or were transferred into the chamber daily for the 7 hour exposure period only. Details concerning the temperature, humidity and photoperiod within the animal room were not supplied. - Animal caging was not described. - Bedding material was not specified. + Dams were allowed to eat and drink *ad libitum* except during the exposure period. - The sources of water and feed were not indicated. - The type of feed was not specified
D. Equipment:	+ A basic description of the exposure chamber and gas delivery system was provided. Further details were found in a companion paper (Laskin and Drew, 1970). + The equipment used to measure the gas concentration and airflow rate was described.
E. Procedural:	- No acclimatization period was reported. - No description of pre-test conditions was provided. - Whether dams were randomly assigned to exposure groups was not noted. + Control animals were reportedly maintained under the same conditions as treated dams. - Unclear whether technicians and handlers were blinded to exposure conditions. - Conventional measures of toxicity, including clinical observations, body weights, pregnancy outcome, clinical pathology and gross pathology, were completely absent - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	+/- Maternal blood was sampled twice, on the day of partuition and day 21pn. Although more measurements would have been desirable, this did permit the reversibility of the changes within this period to be determined. - Individual data were not provided for each test animal, thereby, limiting the independent assessment of the findings. - No numerical data were provided. Results were displayed in a bar graph figure only.
G. Data analysis:	- Data were not statistically analyzed. - Confidence intervals were not provided.

H. Interpretation:	- The lack of detail concerning the number of animals on test renders any interpretation of the findings very difficult. - Many of the investigators' conclusions represent hypotheses only, without supporting empirical data.

Review - Summary:

Discussion of findings:

Exposure of pregnant rats to 50ppm H_2S for 7hr/day from day 6 postcoitus until day 21 postnatal was associated with an average 30% increase in maternal blood taurine levels relative to corresponding control values. According to the authors, the difference between taurine levels in control and treated dams did not increase between birth and day 21, suggesting that taurine levels were elevated before birth and remained elevated until weaning.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- Although the authors suggest that the elevated plasma taurine levels in the dams could have the potential to produce neurological abnormalities in the offspring, the toxicological significance of the findings remains largely unknown. Interpretation of the significance of the findings would have been improved by extending the measurement of taurine levels to the pups borne by the dams as part of the same study. In earlier work, the authors reportedly showed an increase in the levels of taurine in the developing rat CNS. The authors also cite evidence of the transfer of taurine from the dams to the pups both transplacentally and via the milk. However, the exact correlation between plasma levels of taurine in the dam and taurine levels in the offspring has not been established.
- Interpretation of the significance of the findings might have been improved by including some indication as to whether or not any clinical signs of toxicity were expressed by the dams and/or pups during or following the exposures. There is no indication in the report that routine clinical observations of the animals were performed, despite the fact that the exposure chamber was constructed of plexiglass, thereby allowing visual observation of the dams and their litters. Clinical evidence of neurological effects in the pups might have strengthened the authors' claim that the changes in plasma taurine levels in the dams could predispose the offspring to neuronal abnormalities. Without such a connection, the meaning and significance of the changes in taurine levels are questionable. Clinical observations also might have allowed for judgement of the level of maternal stress caused by the exposures, thereby permitting more meaningful interpretation of the findings. It is unknown as to whether the changes in taurine levels represent a direct effect of H_2S or reflect a non-specific response to maternal stress.
- Confidence in the findings and conclusions of the paper is seriously undermined by the lack of detail concerning the number of animals on test. There is no indication of the number of control or treated rats. Accordingly, the strength of the association between the H_2S exposures and the reported changes in plasma taurine levels cannot be established. This further confounds the interpretation of the significance of the findings.
- Any interpretation of the significance of the findings should respect the fact that during the period of organogenesis and early neonatal development, the animal is sensitive to exogenous and endogenous factors that can influence the integrity and function of the CNS and other systems. However, in this instance, further study is needed to substantiate the conclusions of the investigators, which represent hypotheses only and are not supported by empirical data at present.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Lack of information concerning the number of animals on test.
- Lack of basic information concerning animal housing.
- Lack of detail concerning animal handling.
- Lack of statistical analysis of the study findings.
- Failure to include any conventional measures of toxicity, such as clinical observations, as part of the study design.
- Lack of empirical data to support study conclusions concerning impact of maternal plasma taurine levels on pup neuronal development.

Review – Confidence index ranking:

Low to moderate

Document Review

Author:	Curtis, SE; Anderson, CR; Simon, J; Jensen, AH; Day, DL; Kelley, KW	**Internal ID:**	416
Title:	Effects of aerial ammonia, hydrogen sulfide and swine-house dust on rate of gain and respiratory-tract structure in swine.		
Year:	1975		
Abstract:	*"Ammonia, hydrogen sulfide and swine-house dust at continuous aerial levels as high as or higher than those normally encountered in enclosed swine-finishing houses had little or no effect on rate of body-weight gain and respiratory-tract structure of 92 healthy pigs in seven trials. The trials were of 17 to 109 days duration, involved air pollutants alone and in various combinations and were conducted in air-pollutant exposure chambers."*		
Objective:	To investigate the effect of sub-acute exposure to hydrogen sulfide alone or in combination with NH_3, on the rate of bodyweight gain and on the gross and microscopic integrity of respiratory, ocular and visceral tissues of pigs. [NB: The impact of dust and NH_3 alone and in combination was also examined.]		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
(a) 8.5ppm H_2S (b) 2ppm H_2S + 50ppm NH_3	Repeated/ Continuous exposure for… (a) 17 days (b) 19 days	Pig	Crossbred	Not specified.	Not specified.	12. (3 pigs per exposure group).	*"Healthy"*	8.5ppm	NA

Observations:

- Mean-daily body weight gain:
  - 8.5ppm H_2S: 8.6% decrease
  - 2ppm H_2S + 50ppm NH_3: 4% decrease
- Gross and microscopic findings: No evidence of structural alterations in the respiratory tract, eye and its associated structures and the visceral organs.

*Statistically significant

Author's Conclusions:

- *"Hydrogen sulfide, either alone at 8.5ppm or at 2ppm in combination with NH_3 at 50ppm, had little effect on the pigs' growth rate."*
- *"…results indicate that the rate of gain and respiratory-tract structure of growing pigs, which are free of respiratory disease, are not directly influenced by NH_3, H_2S and dust at levels and in combinations commonly encountered in the air inside enclosed houses at commercial swine production operations."*

Review – Assessment:

A. Test animals:	+/- The number of animals on test was somewhat limited (*i.e.*, n= 3 per exposure group). Guidelines for subacute toxicity testing in non-rodent species generally recommend use of 4 animals per treatment level. + Most principle characteristics of the test animals were not defined (*e.g.*, sex, age, pre-test health status and source). +/- The weight of test animals was recorded but information pertaining to weight variation was lacking.
B. Exposure conditions:	- The source of the H_2S gas was not specified. + The duration of exposure was reported. +/- A whole body exposure chamber was used. +/- Pigs were housed in the chamber throughout the entire exposure period. +/- It was unclear whether animals were placed in the exposure chamber before or after the gas was allowed to equilibrate. - Exposure was interrupted daily for 5 min, at which time the cages were cleaned. The time to re-equilibrate the chamber was not indicated. - The numbers of animals placed in a single chamber was not specified, but was assumed to be three. + Airflow and temperature were defined and reportedly satisfied OECD guidelines for these parameters. +/- Humidity was stated to be *"the same as or...lower than that of the outside air"*, but the actual level was not stated. + Gas concentrations were reported to be always within the range of +/- 10% of the desired level. - Pigs were not individually housed.
C. Housing/Feeding:	[NB: animal were housed in exposure chambers] +/- The type of feed supplied (fortified corn-soybean) was reported, but the feeding schedule was not defined. + Details concerning the manner by which water was supplied was reported, but the source and purity of water were not defined.
D. Equipment:	+ The exposure device was adequately described (*i.e.*, material and dimensions). A companion paper with additional detail (Hinners *et al.*, 1968) was cited. +/- The level of H_2S was determined using a colorimetric indicator apparatus. Whether measurements were taken at the animal level was not stated. +/- Flow-meter readings were depended upon for maintenance of the desired level of H_2S in chamber air. +/- The ventilation rate was monitored with a magnahelic gauge attached to the exhaust duct across an orifice plate.
E. Procedural:	- No indication that test animals were quarantined or acclimatized prior to treatment. - No description of pre-test conditions was provided. + Pigs were randomly assigned to exposure groups *"within littermate quartets of the same sex"*. +/- Reference to controls as part of the study, but no details supplied. - Unclear whether technicians and handlers were blinded to exposure conditions. - Whether consideration was given to avoid experimental bias by random scheduling of animal euthanization and necropsy was not noted. - Method of sacrifice was not indicated. - Only two exposure concentrations were investigated (one of which involved H_2S in combination with ammonia). Guidelines generally recommend the use of three graded levels and a corresponding control. A comparison between these concentration levels was further hampered by the addition of ammonia exposure to one group's exposure conditions. - The diet of pigs was altered once a certain total body weight was reached. This dietary change could have affected weight gain. - Interval between euthanization and necropsy was not specified. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.

F. Data collection:	- Details concerning the exact nature of the tissues subjected to histopathological examination were lacking. The report only states that representative samples of the respiratory tract, eyes and viscera were examined. - Individual data were not provided for each test animal, thereby limiting the independent assessment of the findings.
G. Data analysis:	- The type of statistical analysis conducted was not reported. - Confidence intervals were not provided.
H. Interpretations:	- The authors stated that the *"effects of treatment and/or chamber were assumed to be due to treatment alone."*. Whether this was a valid assumption was not investigated.

Review - Summary:

Discussion of findings:

Pigs were exposed to either 8.5ppm H_2S alone or to 2ppm H_2S in combination with NH_3 for 17-19 days under chamber conditions. Treatment was without effect on body weight gain. Necropsy findings were unremarkable. Microscopic examination of the eyes, respiratory tract and viscera showed no changes relative to control animals. Turbinates, trachea and lungs were classified as normal.

Interpretation of the significance of the observed findings should take into consideration the following:

- Limitations in the number of measures of toxicity performed, together with the use of only a single exposure concentration of H_2S alone and use of a restricted number of animals suggests that the study should be considered preliminary in nature. The study could have been improved through performance of routine clinical observations and measurement of routine clinical pathology parameters (*i.e.*, hematology, clinical chemistry, and urinalysis). In addition, interpretation of the body weight gain data would have been improved by provision of absolute body weights and feed consumption data. Better description of necropsy and histopathology procedures would have added to the level of confidence assigned to the study findings and conclusions. Use of graded exposure concentrations would have permitted assessment of dose-responsiveness, which, in this case, would involve the determination of response thresholds as well as the nature and severity of effects at higher exposure levels.
- The absence of histological abnormalities within the respiratory tract (based on examination of turbinates, trachea and lungs) is of interest given the irritant properties of H_2S and the nature of the exposure regimen (*i.e.*, continuous exposure for up to 19 days). Interpretation of the relevance of these findings to the human condition should necessarily consider possible differences in breathing patterns and rates.

Confidence index:

The level of confidence assigned to the study findings and conclusions was undermined by several limitations in experimental design, conduct and/or reporting. The limitations included:

- Use of limited number of test animals.
- General lack of information concerning test animals.
- Use of a single exposure concentration of H_2S alone.
- Lack of most routine measures of toxicity performed as part of subacute toxicity studies.
- Inadequate reporting of histopathological procedures and findings.
- Lack of confidence in accuracy and reliability of "*colorimetric indicator apparatus*" to measure H_2S concentration.

Review – Confidence index ranking:

Low

Document Review

Author:	Alberta Social Services and Community Health	**Internal ID:**	424
Title:	Lodgepole gas well blowout, 1982: A report on the hydrogen sulphide concentrations, frequency and nature of complaints. Oct. 31, 1983.		
Year:	1983		
Abstract:	*Not applicable*		
Objective:	The report aims to analyze the nature and frequency of complaints filed with Alberta Environment during the Lodgepole gas well blowout and to compare these with the hydrogen sulfide concentrations monitored during this incident.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
(a) Edmonton: 0ppm to 0.521ppm (b) Calgary: 0ppm to 0.328ppm (c) Northern rural area: 0ppm to 5.5ppm (d) Southern rural area: 0ppm to 2.8ppm	Phase I: 14 days Phase III: 10 days	Not specified.	Not specified.	Not specified.	Not specified.	NA	NA

Observations:

- Overall 1477 complaints were received
  - Type of complaints:
    - General (*i.e.*, detection of odor or inquires about the situation): 66.8%
    - Health related (*i.e.*, reports of specific symptoms or just 'feeling sick'): 33.2%
  - Most common symptoms in descending order of occurrence: feeling sick, headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat/cough, vomiting, stomach cramps, skin allergies.
  - Other symptoms of limited occurrence included chest pain, dizziness, insomnia, nose bleeds, tiredness, loss of appetite, voice problems, numbness, suspected heart attack, backache, tingling limbs, abdominal pains, loss of hair, double vision, dry mouth, loss of weight, loss of sense of smell, high blood pressure, ruptured septum and emotional disturbances.
  - Frequency of complaints:
    - Phase I (well unlit): 26.2% of complaints
    - Phase II (well lit): 2.6% of complaints
    - Phase III (well unlit): 62.8% of complaints
    - Phase IV (well lit): 8.4% of complaints

- Edmonton (1187 complaints received):
  - Type of complaints:
    - General: 64.8%
    - Health related: 35.2%
  - Frequency of complaints:
    - Phase I: 21.7%
    - Phase II: 1.6%

- Phase III: 67.9%
- Phase IV: 8.8%

- Calgary (27 complaints received):
  - Type of complaints:
    - General: 70.4%
    - Health related: 29.6%
  - Frequency of complaints:
    - Phase I: 3.7%
    - Phase II: 29.6%
    - Phase III: 40.8%
    - Phase IV: 25.9%

- Northern Rural Area (231 complaints received):
  - Type of complaints:
    - General: 76.6%
    - Health related: 23.4%
  - Frequency of complaints:
    - Phase I: 51.5%
    - Phase II: 3.9%
    - Phase III: 37.7%
    - Phase IV: 6.9%

- Southern Rural Area (32 complaints received):
  - Type of complaints:
    - General: 65.6%
    - Health related: 34.4%
  - Frequency of complaints:
    - Phase I: 28.1%
    - Phase II: 6.3%
    - Phase III: 65.6%
    - Phase IV: None

*Statistically significant

Author's Conclusions:

- *"From the 491 health related complaints received, 31 symptoms were identified."* The symptoms experienced most frequently included feeling sick (21.2%), headache (16.1%), nausea (13.8%), diarrhea (9.9%), respiratory problems (7.6%), burning eyes (6.5%), sore throat (5%), vomiting (4.3%), stomach cramps (4.1%), skin allergies (2.3%).
- *"The majority of complaints were received when the well was not on fire."*
- *"The frequency of complaints appeared to vary as the concentration of hydrogen sulfide gas in the environment, although some inconsistencies were observed. The maximum frequency of complaints coincided with the maximum hydrogen sulphide concentration (daily average or peak) only in the Calgary and Southern Rural area."*

Review – Assessment:

A. Subjects:	- Only the number of complaints were recorded. The number of individuals who lodged these complaints was not noted. - The age, sex, health, work history and medical history of those filing complaints were not reported.
B. Exposure conditions:	+ The H_2S concentration was monitored at six stations. - The precise concentration and duration of exposure to which individual residents were exposed is unknown. - It is unknown how close the monitoring stations were to residential areas. - The identity and concentration of other released substances were not reported.
C. Equipment:	- The nature of the monitoring equipment (*e.g.*, type, detection limit, etc.) was not described.
D. Procedural:	- The symptoms were not confirmed by medical practitioner(s). - The frequency of symptoms in a control community was not assessed. - Blinding was not possible + Complaints were filed during the incident, thereby eliminating the potential for recall bias. - No follow-up was conducted.
E. Data collection:	- Complaints were self-reported. - The nature of the questions posed by individuals manning telephone lines was not specified. - As noted by the author *"some caution should be taken in interpreting the frequency and nature of complaints due to the large number of telephone calls received simultaneously."* - Although records of complaints since October 17th were included in this report, the telephone number for Alberta Environment was distributed via Edmonton media on November 24th. - The severity and reversibility of symptoms was not recorded. - Individual data were not provided, therefore, independent analysis of results is limited.
F. Data analysis:	- Beyond the calculation of the mean and standard deviation, no statistical analyses were conducted.
G. Interpretations:	

Review - Summary:

Discussion of findings:

Chronologically the Lodgepole incident can be divided into four distinct exposure phases. During phase I (October 17th to 31st) and III (November 15th to 25th) the well was not ignited. With the exception of the Calgary region, it was during these periods when the highest frequency of health complaints, from local residents, were lodged with Alberta Environment. However, peak daily averages only coincided with the maximum frequency of complaints in Calgary and the Southern rural region. Thirty one distinct symptoms were reported including general malaise, headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, vomiting, stomach cramps and skin allergies.

Interpretation of the significance of the study findings and their relevance to the general population should consider the following:

- Symptoms were self-reported and not verified by medical practitioners.
- It is unknown what percent of the exposed population experienced symptoms.
- A baseline of symptom frequency in an unexposed population was not provided.
- The precise location of monitoring devices with respect to residential areas was not noted. Thus, the actual concentration to which residents were exposed is unknown.
- Comparisons between complaint frequency and H_2S levels commenced in mid-October, however, the telephone number for Alberta Environment was

distributed by media in Edmonton on November 24th. This publicity may have skewed results. It was on this day, that the maximum number of complaints in Edmonton were filed.

Confidence index:

The level of confidence that could be assigned to reported findings and conclusions was deemed to be low. Several weaknesses in the investigation and reporting were evident. The weaknesses included:

- Lack of information pertaining to residents who filed complaints.
- Unknown reliability of exposure estimates.
- Possible subject bias.
- Absence of medical data to confirm the reported symptoms.
- Failure to account for chemical exposures apart from H_2S that may have contributed to the observed symptoms.

Review – Confidence index ranking:

Low to moderate

Document Review

Author:	Mitchell, CW; Yant, WP	**Internal ID:**	444
Title:	Correlation of the data obtained from refinery accidents with a laboratory study of H_2S and its treatment.		
Year:	1925		
Abstract:	*"In the laboratory study, the symptoms of hydrogen sulphide (H_2S) poisoning in animals and men were found to be almost identical with those caused by gases in the refineries. The need for a definite method of treating H_2S poisoning was evident. The medical findings, the study on toxicity of H_2S, and the treatment for H_2S poisoning will be discussed in turn."*		
Objective:	To investigate the toxicity of hydrogen sulphide in various laboratory animal species as a possible means to further understanding of the onset, progress and treatment of H_2S poisoning among refinery workers. The animal species tested were canary birds, rats, guinea pigs, dogs and goats. Canaries were chosen because of their susceptibility to poisonous gases, and goats for their resistance. Preliminary studies involving exposure of human subjects to H_2S under controlled conditions were also performed..		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
35 to 1600 ppm[1] ("whole body")	Single exposures lasting up to 100 hours, depending on species	(a) Canary (b) Rat (c) Guinea pig (d) Dog (e) Goat (f) Human	Not specified	Not specified	Not specified for animal species. Male subjects were used in the human study	(a) 8 exposed (b) 23 exposed (c) 4 exposed (d) 2 exposed (e) Unknown	Stated to be "healthy"	(a) 35 ppm for up to 4hours (b) 35 ppm for up to 1 hour (c) 35 ppm for up to 8 hours (d) 103 ppm for up to 4 hours (e) 820 ppm for up to 2 minutes (f) 100 ppm for up to 2 minutes	(a) 35 ppm for longer than 4 hours (b) 35 ppm for longer than 1 hour (c) 35 ppm for longer than 8 hours (d) 103 ppm for longer than 4 hours. (e) 820 ppm for longer than 2 minutes (f) 100 ppm for longer than 2 minutes

1. Exposure concentrations up to 100 ppm are emphasized as part of this review.

Observations:

- Canaries
  - 35ppm to 65 ppm:
    - 0 to 4 hour exposure: No symptoms
    - 4 to 8 hour exposure: "Stupid"; heavy breathing; edema
    - 8 to 18 hour exposure: Weakness; death
  - 97ppm to 100 ppm:

    - 0 to 30 minute exposure: No symptoms
    - 30 minute to 1 hour exposure: One case of slight edema; one case of panting
    - 1 to 4 hour exposure: Edema; dizziness; general stupidity; forced breathing through mouth
    - 4 to 8 hour exposure: Very forced respiration; edema; dizziness; unconsciousness and death
    - All the birds that did not die during the experiment died 12 to 36 hours later

- Rats
  - 35ppm to 65 ppm:
    - 0 to 1 hour exposure: No symptoms
    - 1 to 4 hour exposure: Animal continually washing face
    - 4 to 8 hour exposure: Irritation of nose and eyes
    - 8 to 18 hour exposure: "Quiet"
    - 18 to 48 hour exposure: Pus in eyes and nose; hair rough
  - 100 ppm:
    - 0 to 1 hour exposure: No symptoms
    - 1 to 4 hour exposure: Animal continually washing face
    - 4 to 8 hour exposure: Irritation of nose and eyes
    - 8 to 18 hour exposure: Forced breathing
    - 18 to 48 hour exposure: Death

- Guinea pigs
  - 35 to 65 ppm:
    - 0 to 8 hour exposure: No symptoms
    - 8 to 18 hour exposure: Hair rough
    - 18 to 48 hour exposure: Hair rough; pus in eyes and nostrils; cough
  - 103 ppm:
    - 0 to 8 hour exposure: No symptoms
    - 8 to18 hour exposure: Labored and forced breathing
    - 18 to 48 hour exposure: Death in one case.

- Dogs
  - 103 ppm:
    - 0 to 4 hour exposure: No symptoms
    - 4 to 8 hour exposure: Lachrymation; depression
    - 8 to 16 hour exposure: Pus in eyes; death

- Goats
  - Lowest exposure concentration tested was 820 ppm

- Humans
  - 100 to 150 ppm:
    - 0 to 2 minute exposure: No symptoms
    - 2 to 15 minute exposure: Coughing; eye irritation; loss of sense of smell
    - 15 to 30 minute exposure: Disturbed respiration; pain in eyes; sleepiness
    - 30 to 1 hour exposure: Throat irritation

- 1 to 4 hour exposure: Salivation and mucous discharge; sharp pain in eyes; coughing
*Statistically significant

Author's Conclusions:

- *"...The canaries are a great deal more susceptible than rats; the rats but little, if any more, than dogs; the dogs decidedly more than guinea pigs; and guinea pigs but little more than goats."*
- *"The lowest concentration of H_2S to which men were subjected was 0.01 per cent, which was definitely irritating. From the experiments with canaries and animals, symptoms of irritation were observed in 0.005 per cent, and in all probability further experiments will demonstrate that exposures to even lower concentrations over long periods will cause poisoning."*
- *"This study on toxicity demonstrates that animals of the types and sizes studied are quite susceptible to H_2S poisoning, and that these animals show very similar symptoms. These symptoms are comparable to those in men exposed to low concentrations, and to those reported as occurring in accidents from H_2S poisoning in industrial plants."*
- *"Acute poisoning can be produced by concentration of 0.06 to 0.08 per cent or more of H_2S."*
- *"Subacute poisoning can be produced by long exposures to concentrations as low as 0.005 per cent."*
- *"Pathological findings are typical of irritation. Microscopic examination indicates destruction of cell lining of lung alveoli".*
- *"In acute cases, if there was recovery, it was rapid, and only rarely were any after effects apparent for more than a few hours; but in the subacute cases, the recovery was slow, sometimes taking several days".*
- *"The symptoms of irritation from subacute poisoning usually last from one to seven days, their duration depending on the severity of the irritation produced".*

Review – Assessment:

A. Test animals:	- The number of treated/control animals was limited and number of treated/control human subjects was not stated. - Principle characteristics of exposed animals and subjects were not defined beyond the statement that they *"all were healthy and representative of their kind."* - The source of the test animals was not indicated and the manner by which human subjects were recruited was not stated. - It is unknown whether human subjects provided informed consent.
B. Exposure conditions:	- The manufacturer and purity of the FeS and HCl used to generate the H_2S was not reported. - The precise exposure concentration(s) was not stated. Only a range was quoted. +/- The duration of exposure was defined; however, only time intervals were listed for the reporting of signs and symptoms. + A whole body exposure chamber was used. + Animals/subjects were placed in the exposure chamber after the equilibration period. - Whether oxygen content, pressure, humidity, and photoperiod were monitored throughout exposure was not reported. + The distribution of the gas within the chamber maintained through the use of a fan to ensure homogeneous mixing. +/- At intervals during the exposure period, the H_2S concentration in the chamber was determined by the cadmium chloride method. How sampling was performed without altering the concentration within the chamber was not specified. +/- Exposures were stated to be "continuous"
C. Housing/Feeding:	- Details concerning animal housing (*i.e.*, temperature, humidity, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type and dimensions) was not provided.

	- Bedding material was not specified. - The type and source of feed was not reported. The feeding schedule was also omitted. - Details concerning the water supply were lacking.
D. Equipment:	- Exposure was completed in a 1000-cubic foot gas chamber. +/- Description of the exposure chamber was limited. Further description is provided in US Public Health Reports, vol. 37(19), May 12, 1922 pp.1127-1142, which was not readily available. +/- A Kipp generator was employed to generate the H_2S gas. - The cadmium chloride method was used to measure the gas concentration within the chamber. The method was judged to provide limited sensitivity.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. - Detail pertaining to the randomization of test animals and assignment to test groups was lacking. +/- Control experiments were conducted in pure air. +/- Following death, a pathological examination was made for gross changes and specimens of the lungs, heart, liver, and kidneys were microscopically examined. +/- All symptoms were noted, as well as, the time of occurrence. However, only a time range was provided. +/- Study pre-dated Good Laboratory Practice (GLP) guidelines. - Unclear whether technicians and handler were blinded to exposure conditions.
F. Data collection:	+/- General comments on reversibility of symptoms were made; however, they were not specific to each symptom observed. - Individual data were not provided for each test subject/animal, thereby limiting the independent assessment of the findings. - Other than the statement that control results was *"negative,"* no further data were provided. - All observational data was generalized in tables. - Necropsy and histology data were not provided for exposure levels up to 350ppm. - The number of animals in which the symptoms were noted was not recorded.
G. Data analysis:	- Data were not statistically analyzed.
H. Interpretations:	- The authors believed that based on the results of human exposure trials up to 100ppm for 4 hours and data from canine studies, it is possible to predict the reaction of men to higher concentrations. The validity of this statement is questionable, as it is unclear whether issues of "toxic load" were considered. - The human studies were described as "preliminary".

Review - Summary:

Discussion of findings:

Large variations in specie sensitivity to H_2S intoxication were demonstrated in experiments of whole body exposure of canaries, rats, guinea pigs, dogs, goats and humans. The duration of exposure appeared to have a significant influence on the type and severity of symptoms observed., with most symptoms progressing with continued exposure. Signs and symptoms were also influenced by the exposure concentration.

Deaths of canaries was observed following 8 to 18 hours exposure to 35 to 65ppm H_2S, whereas rat deaths were noted following a 18 to 48 hour exposure to 100ppm H_2S. Similarly, guinea pig and dog mortality was noted following exposure to 103ppm H_2S for a period of 8 to 48 hours and 8-16 hours respectively.

Symptoms of respiratory distress, depression, eye/nose and throat irritation were observed following shorter exposure periods.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The study is dated and was performed long before the development of testing guidelines and the introduction of Good Laboratory Practice (GLP) requirements. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested.
- Exposure concentrations and the time of appearance of symptoms were reported only as ranges. This hinders interpretation of the dose-responsiveness and time-responsiveness of the findings.
- The number of animals on test was limited. In most instances, group sizes were limited to 1 to 4 animals per specified range of exposure concentrations. Group sizes were below those recommended by testing guidelines.
- No pre-trial health examinations were conducted. Given the age of the study, and the general health of animals and animal care practices in place at the time, the possibility is presented that certain of the responses (especially the pathological findings) may have been non-treatment-related.
- The number of control animals/subjects included in the study was not specified.
- Apart from the preliminary human study, the sex of the test animals was not specified. Only male subjects were used in the human study.
- With the exception of symptoms observed in canaries following 30 minutes exposure to 97 to 100ppm H_2S, the number of exposed animals exhibiting the observed symptoms was not reported.
- No necropsy or histopathological data were provided for the animals exposed to the lower exposure concentrations (*i.e.*, <103 ppm).
- Overall lack of detail pertaining to procedures (*e.g.*, blinding, randomization), equipment and animal/subject characteristics (*e.g.*, pre-trial health, breed, sex, age) undermines the level of confidence that can be assigned to the study findings and conclusions.
- The responses noted among the canary birds may not be representative since the authors contended that canaries are "*extremely sensitive*" to poisonous gases.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable weaknesses included:

- Use of very limited numbers of test animals.
- Failure to specify the number of control animals/subjects included in the studies.
- Lack of detail concerning exposure conditions, the exposure chamber, and gas delivery system.
- Complete lack of detail concerning animal housing and husbandry.
- Lack of details concerning randomization and assignment of test animals to groups, and blinding of subjects/technicians to exposure conditions.
- Failure to specify the sex of test animals.

Review – Confidence index ranking:

Low

Document Review

Author:	Hulbert, WC; Yang, ZJ; Trebilcock, MK; Donelon, J; Boyd, WA	**Internal ID:**	460
Title:	Effects of hydrogen sulphide on the pulmonary system.		
Year:	1989		
Abstract:	*Not available*		
Objective:	To examine the response of pulmonary tissues to exposure to H_2S in guinea pigs "acute" through assessment of baseline pulmonary function, bronchial reactivity to inhaled methacholine, and lung tissue morphology using both light and scanning electron microscopy. N.B. The study consisted of two separate experiments involving different exposure concentrations and exposure times. Experiment (a) investigated the effect of "acute" exposure to H_2S at moderately high concentrations (100ppm and500ppm).Experiment (b) examined the effect of sub-chronic exposure to 1 to 100ppm H_2S. Only Experiment (a) was subjected to detailed review.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health status	NOAEL	LOAEL
0 ppm; 100ppm; 500ppm (intratracheally)	Single/ 1 hr	Guinea pig	Cam-Hartley	Not specified.	Not specified.	Not specified.	Not specified.	NA	100ppm for 1 hour.

Observations:

- 100ppm H_2S:
  - Airway resistance (R_L): 10-fold decrease following 10min of exposure; return to pre-H_2S exposure level by 30min, indicating bronchodilatory event.
  - Dynamic compliance (C_{dyn}): 10-fold increase following 10min of exposure; return to pre-H_2S exposure level by 30min.
  - Tidal volume (V_E): Varied slightly.
  - Tidal frequency (f): Varied slightly.
  - Tidal minute volume (V_T): Varied slightly
  - Responses applied to *"approximately 50% of the animals"*.

- 500 ppm H_2S: No results reported.

- 100ppm H_2S+ inhaled Methacholine:
  - Airway resistance (R_L): No change in dose-response curve.
  - Dynamic compliance (C_{dyn}): Shift of dose-response curve to right.

- 500ppm H_2S + inhaled Methacholine:
  - Airway resistance (R_L): 10-fold left-shift in dose-response curve, indicating increased sensitivity.
  - Dynamic compliance (C_{dyn}): 10-fold left shift in dose response curve.

- 100ppm or 500ppm H_2S + I.V. Methacholine:

  - Airway resistance (R_L): No change in dose-response curve.
  - Dynamic compliance (C_{dyn}): No change in dose response curve.

- Microscopy:
  - 100ppm H_2S: No change
  - 500ppm H_2S: Formation of small blebs on the bronchiolar ciliated epithelial cells. Otherwise tissues were reported to be *"normal"*.

*Statistically significant

Author's Conclusions:

- *"…100ppm H_2S, inhaled for 1 h, causes transient bronchodilation in some individuals."*
- *"H_2S may act by hyperpolarizing the irritant receptors and blocking parasympathetic transmission…effects are reversible…Membrane hyperpolarization, like a buildup of acetylcholine, will also block cholinergic tone, causing the airway smooth muscle to dilate."*
- *"The increased sensitivity to inhaled Methacholine but not iv Methacholine in the animals exposed to 500ppm is significant. This implies that the mechanism of altered sensitivity does not lie at the level of the smooth muscle, rather it is at the level of the epithelium."*
- *"…by comparison, the cytotoxic effects of H_2S are minimal compared to 0_3 or even $S0_2$."*

Review – Assessment:

A. Test animals:	- The number of test animals used in the "acute" study was not specified. + The specie and strain of test animals was reported. - The pre-test health status, age, sex, weight variation and source of animals was not defined.
B. Exposure conditions:	- The source of H_2S gas was not reported. + The duration of exposure was precisely defined. + Experiments were designed *"to simulate a mouth-breathing condition"*. Guinea pigs were anesthetized, tracheostomized and mechanically ventilated.
C. Housing/Feeding:	+ Animals were reared in *"Bioclean Porta-rooms"* which were equipped with laminar air flow and HEPA filtering. - Details concerning environmental conditions within the animal room (*i.e.*, temperature, humidity, and photoperiod) were lacking. + Animals were individually caged. +/- The material from which cages were constructed was noted but the dimensions were not included. - Bedding material was not specified. - The type and source of feed supplied was not recorded. - Details concerning water supply were lacking. - The nature of the feeding schedule was not addressed (*e.g.*, *ad libitum*)
D. Equipment:	+ A schematic of gas delivery system was provided. - The manner by which gas concentration, airflow rate, humidity, temperature, and oxygen levels were monitored was not described.
E. Procedural:	- No acclimation period was reported. - A description of pre-test conditions was lacking. - No indication that study was performed under Good Laboratory Practice (GLP) conditions. - Only two exposure concentrations were tested, thereby limiting examination of dose-responsiveness.

F. Data collection:	- No numerical data were presented. Results were presented in figures only, of limited resolution. - A complete set of figures was not provided. (Figures appeared to be "representative" only, with only one animal included). +/- Representative photomicrographs of pulmonary tissues were provided.
G. Data analysis:	- No indication that findings were subjected to statistical analysis. - Confidence intervals were not provided.
H. Interpretations:	+ The authors placed the findings into perspective by comparing the morphological changes to those observed following exposure to other oxidative gases. - Mode of exposure was "artificial" involving intratracheal treatment with mechanical ventilation.

Review - Summary:

Discussion of findings:

One-hour exposure to 100ppm H_2S reportedly produced in a transient 10-fold change in airway resistance and dynamic compliance in treated guinea pigs, marking *"an acute bronchodilatory effect"*. Variations in tidal volume, frequency and minute volume were reported to be minimal. Concurrent inhalation of methacholine did not impact the airway resistance dose-response curve, but produced a right shift in dynamic compliance of unknown significance. The effect of methacholine inhalation was more marked when introduced concurrently with 500ppm H_2S. A 10-fold left shift of dose-response curves was observed for both airway resistance and dynamic compliance, indicative of heightened sensitivity. Intravenous administration of methacholine directly into smooth muscle had no effect on the dose-response curve, suggesting that the observed heightened sensitivity lies at the level of the epithelium, and not the smooth muscle.

Microscopic examination of pulmonary tissues exposed to 100ppm H_2S revealed no apparent damage. At 500 ppm, mild cytotoxicity was expressed in the form of minor "blebs" on the bronchiolar ciliated epithelial cells.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The mode of administration of the H_2S involved intra-tracheal treatment of the animal under restraint and aided by mechanical ventilation. The aim was to simulate a "mouth-breathing condition", whereby the gas was delivered directly to the lungs, bypassing the "scrubbing" action provided by the mucosal epithelium lining the upper respiratory passages. The condition is largely "artificial", rendering extrapolation of the findings to the human condition difficult. Apart from individuals engaged in strenuous physical activity (in which mouth breathing would predominate), the findings likely over-state the changes that might occur in the pulmonary tissues of a person.
- The only significant change observed following the one-hour exposure to 100 ppm of H_2S was an instantaneous *decrease* in airway resistance and *increase* in dynamic compliance. Consistent with the authors' suggestion, this type of change signifies a "bronchodilatory event", which is contrary to the response that might be expected given the irritant properties of H_2S and the more commonly reported *broncho-obstruction* following exposure.
- The changes in pulmonary function (*i.e.*, reduced airway resistance and increased compliance) evidently were observed in only 50% of the test animals, and were not accompanied by any histopathological evidence of damage to the pulmonary tissues.
- A general lack of detail surrounds the description of methods and results from the study, which seriously undermines the level of confidence that can be assigned to the findings and conclusions. For example, the number of test animals was not specified, the sex of the test animals was not indicated, only selected data were reported, and no statistical analyses were performed On the basis of the information provided, it is very difficult to assign significance to the findings.
- The study lacked routine measures of toxicity (*i.e.*, body weights, clinical observations, gross pathology) that might have improved interpretation of the significance and relevance of the findings and conclusions.

Confidence index:

Confidence in the study findings and conclusions was seriously undermined by numerous weaknesses in experimental design, conduct and/or reporting. The limitations included:

- Unspecified number of test animals.
- General lack of information concerning test animals and housing conditions.
- Use of a limited number of exposure concentrations.
- Presentation of results exclusively in the form of figures of limited resolution, with no numerical findings provided.
- Failure to limit potential confounding variables by study design or statistical analysis.
- Failure to include conventional measures of toxicity as part of the overall study design.

Review – Confidence index ranking:

Low

Document Review

Author:	Lopez, A; Prior, M; Leblanc, D; Young, S; Al-Bassam, M; Lillie, L.E.	**Internal ID:**	466
Title:	Morphological observations in rats exposed for six hours to an atmosphere of 0, 56, or 420mg/m^3 hydrogen sulfide.		
Year:	1986		
Abstract:	*"Forty eight male Long Evans rats were exposed to nominal concentrations of 0, 56 or 420 mg m^{-3} (actual 0, 57 ± 15 or 420 ± 1.4 mg m^{-3}) hydrogen sulphide for six hours. Weight loss was observed in all rats exposed to hydrogen sulphide, as was agitation, hypoaesthesia, panting and lacrimation. All rats exposed to 300 ppm died within the six hour exposure period. Necrosis of the nasal epithelium was more marked in the intermediate (sectors 2, 3) than the most rostral (sector 1) and most caudal (section 4) parts of the nasal cavity. The lateral aspects of the nasal turbinates revealed more necrosis when compared to the median aspects, especially the epithelium covering the nasal septum. Mild pulmonary oedema was observed in all animals exposed and killed by 420 mg m^{-3} hydrogen sulphide and in those treated with 56 mg m^{-3} and killed at the end of the exposure. Rats exposed to 56 mg m^{-3} did not show pulmonary oedema at 18 or 42 hours post exposure. The oedema had a perivascular distribution, and fluid was rarely seen within the alveoli."*		
Objective:	To examine the initial histological lesions in rats exposed to a 40ppm or 300ppm H_2S for 6 hours, with particular emphasis on the lesions observed in the nasal mucosa.		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm (room control); 0ppm (chamber control); **40**ppm; 300ppm (whole body)	Single/ 6 hours. (Animals were sacrificed at 0, 18 and 42 hours post-exposure).	Rat	Long Evans	Not specified. (Suspected to be juvenile rats based on initial body weight data).	Male	48 total. (12 rats per exposure group)	Not specified	NA	40ppm

Observations:

- Clinical signs:
  - 0 ppm (room and chamber controls): No significant clinical signs.
  - **40**ppm: Initial hyperactivity, lacrimation and moderate respiratory distress for up to 2 hours post-exposure.
  - 300ppm: Hyperactivity, lacrimation, severe respiratory distress until death.

- Mortality:
  - 0 ppm (room and chamber controls): No deaths.
  - **40**ppm: No deaths either during or post-exposure.
  - 300ppm: All rats died close to the end of the 6-hour exposure period (*i.e.*, after 5 hours).

- Body weights:
  - 0 ppm (room control): No data available.
  - 0ppm (chamber control): Initial weight loss immediately following exposure (<5%). Growth restored within 18 hours post-exposure.
  - **40**ppm: Initial weight loss immediately following exposure (<10%). Growth restored within 18 to 42 hours post-exposure.
  - 300ppm: Weight loss recorded at time of death (<10%).

- Gross pathology:
  - 0 ppm (room and chamber controls): No remarkable findings.
  - **40**ppm: No macroscopic lesions.
  - 300ppm: Froth in the upper airways; lungs markedly congested, haemorrahagic and failed to collapse when the thoracic cage was opened.

- Histopathology:
  - 0ppm (Room control):
    - Mild to moderate lymporrhexis in the thymus or lymph nodes (4 of 12 rats affected).
    - Mild to moderate focal rhinitis (3of 12 rats affected)
  - 0ppm (Chamber control):
    - Moderate to severe lymporrhexis in the thymus or lymph nodes (3 of 12 rats affected)
    - Mild focal rhinitis (1 of 12 rats affected)
  - **40**ppm:
    - Mild to severe necrosis of nasal epithelium, especially of lateral aspects within intermediate sectors of nasal cavity. Evidence of reparation by 42 hours post-exposure.
    - Mild pulmonary oedema immediately post-exposure. Signs cleared within 18 hours post-exposure (4 of 12 rats affected).
    - Mild to severe lymporrhexis in the thymus or lymph nodes (7 of 12 rats affected).
  - 300pmm:
    - Moderate to severe necrosis of nasal epithelium, especially of lateral aspects of intermediate sectors of nasal cavity (12 of 12 rats affected).
    - Mild pulmonary oedema (12 of 12 rats affected).
    - Fibrin-like fibrils in alveoli and cellular desquamation from the bronchiolar walls.
    - Mild to severe lymporrhexis in the thymus or lymph nodes (11 of 12 rats affected).
  - All exposure concentrations:
    - Several incidental findings unrelated to treatment.

*Statistically significant

Author's Conclusions:

- *"Weight loss was observed in all rats exposed to hydrogen sulphide, as was agitation, hypoaesthesia, panting and lacrimation."*
- *"evidence of corneal or conjuctival abnormalities were not observed in rats for up to 42 hours after exposure to 56mg m^{-3} for six hours. The lack of ocular lesions…was likely dose or time related since severe eye irritation was clinically observed during and after hydrogen sulphide exposure…lacrimation was noticeable at 56mg m^{-3}"*
- *"…in spite of the highly irritative properties of hydrogen sulphide, the survivors fully recover, without any noticeable residual signs of toxicity."*
- *"Necrosis of the nasal epithelium reflected severe toxicity…to the upper respiratory epithelium even when the concentration was as low as 56mg m^{-3}." "Necrosis of the nasal epithelium was more marked in the intermediate (sectors 2, 3) than the most rostral (sector 1) and most caudal (sector 4) parts of the nasal cavity." "The reason why the most rostral and caudal sectors, as well as the medial compared to the lateral aspects of the nasal cavity were less affected by hydrogen sulphide requires further study." "…the lateral aspects of the nasal turbinates revealed more necrosis when compared to the median aspects, especially the epithelium covering the nasal septum."*
- *"The presence of foamy fluids in the lungs, trachea and nasal cavity of rats exposed to 420mg m^{-3}, and mild histologic accumulation of fluid in the lungs of those animals exposed to 56mg m^{-3} and sacrificed at zero hours post exposure, were undoubtedly good indicators of the oedematogenic properties of hydrogen sulphide on the respiratory tract." "Whether or not pulmonary oedema in individuals exposed to hydrogen sulphide is neurogenic, inflammatory*

or cardiogenic in origin or a combination of all three, remains to be elucidated."

- *"The dose-lesion relationship and sequence of events of the effect of hydrogen sulphide on the respiratory epithelium requires further work".*
- *"Lymphoid cell necrosis was possibly a stress-induced lesion..."*

Review – Assessment:

A. Test animals:	+/- The number of rats allocated to each exposure group and sampling time (*i.e.*, n=4) was somewhat limited. Testing guidelines generally recommended 5 rats per sex/group. - Only male rats were used. Testing guidelines generally recommend use of both sexes. No rationale was provided to justify the use of the male sex only. +/- The principle characteristics of the test animals (*e.g.*, specie, sex, weight and source) were reported; however, information pertaining to age and pre-test health status was lacking. + Weight variation at study initiation was in compliance with guideline recommendations.
B. Exposure conditions:	+/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure. + The gas exposure system was adequately described. + The actual (nominal) gas concentrations were recorded. Gas concentrations were monitored 4 times an hour and analyzed by gas chromatography. + The exposure chamber was maintained at negative pressure, in compliance with guideline recommendations. +/- Pressure and air flow within the exposure chambers were specified, but details concerning temperature and humidity were lacking.
C. Housing/Feeding:	+ Details concerning animal caging were provided (*i.e.*, stainless steel, wire meshed bottomed). + Test animals were individually housed, therefore, clear observation of each animal was possible. + Commercial pelleted rat feed and water were fed *ad libitum* to test animals in accordance with guideline recommendations. + The source of the feed was indicated. Water was stated to be *"reverse osmosis"*. - Feeding restrictions imposed during exposure were not noted. + Animals were housed in an environmentally-controlled room (*e.g.* 22+/- 2°C; 50 +/- 20% humidity; monitored photoperiod) + Airflow was recorded and complied with OECD guidelines (*i.e.*, 15 changes per hour).
D. Equipment:	+ The exposure chamber, gas delivery system and monitoring equipment were adequately described. + Analysis equipment was housed in a room separate from the exposure chamber to prevent cross contamination.
E. Procedural:	+ Animals were acclimatized for two weeks prior to exposure, consistent with guideline recommendations. + Acclimatization to the noise and confinement within the exposure chamber was conducted for 6 hours per day for four days prior to exposure. - Pre-test health including quarantine, and disease treatment were not discussed. +/- Rats were randomly allocated to exposure groups, but the method of randomization (*e.g.* table of random numbers, computer generated) was not noted. - Whether rats were randomly assigned to cage position within the gas chamber was not reported. + The experiment included two control groups. One was a 'room control' and the other was a 'chamber control'. The latter accounted for responses associated with any stress associated with confinement in the exposure chamber. - No indication that study was performed under Good Laboratory Practice (GLP) conditions. - Unclear whether technicians and handlers were blinded to exposure conditions.

	+ Conventional measures of toxicity, including clinical observations, weekly body weight determination, and gross and histopathology were performed. Twenty tissues were processed for microscopic examination. Routine clinical pathology and organ weight measurements were not performed. + Guidelines of the Canadian Council of Animal Care (1980) were followed.
F. Data collection:	+ Nature, severity and duration of clinical signs were recorded. +/- Individual animal data were provided for histopathological changes. Summary data were provided for all other endpoints.
G. Data analysis:	- No statistical analyses were conducted.
H. Interpretations:	+/- Rats are obligate nasal breathers. Accordingly, evidence of necrosis of epithelium lining nasal passages may be more severe than that which might occur in other species. +/- Lymphorrhexis may have been stress-induced.

Review - Summary:

Discussion of findings:

Exposure of male rats to 40ppm H_2S for 6 hours under controlled conditions resulted in initial weight loss and transient clinical signs of intoxication, including agitation, lacrimation and moderate respiratory distress. No animals died. Clinical signs generally resolved within a few hours post-exposure. No macroscopic lesions were observed upon necropsy performed at 0, 18 or 42 hours post-exposure. Histopathologic alterations included focal necrosis of the nasal epithelium, primarily in the intermediate (sectors 2, 3) parts of the nasal cavity, with evidence of repair by 42 hours post-exposure. The reasons for sector specific damage and the pathogenesis of the observed changes were not obvious. Mild pulmonary oedema was observed in the rats killed immediately following exposure. No evidence of pulmonary oedema was found in the rats sacrificed at 18 and 42 hours post-exposure. Despite the lacrimation which presented clinically, no corneal or conjunctival abnormalities were noted upon histopathologic examination of the eyes.

Within the 6-hour exposure period, all rats exposed to 300ppm H_2S died. Clinical signs of intoxication included severe agitation and labored breathing until death. Necropsy revealed froth in the upper airway, lung congestion and pulmonary hemorrhages. Histopathologic examination showed lesions similar to those observed at 40 ppm, but in a more severe form.

Interpretation of the significance and relevance of the study findings to the human condition should take into consideration the following:

- Responses were clearly dose-related, with more severe changes in clinical and pathological outcomes observed in the rats exposed to 300 ppm H_2S compared to 40 ppm.
- Responses were consistent with the known irritant properties of H_2S. The observed lacrimation, focal necrosis of the nasal epithelium, and pulmonary oedema are all indicators of irritation, especially of the mucosa.
- The responses noted at 40 ppm H_2S were transient and generally resolved within several hours post-exposure. Clinical signs were absent within 2 hours after exposure, and no evidence of pulmonary oedema was present at 18 hours post-exposure. Repair of the focal necrosis of the nasal epithelium was obvious at 42 hours post-exposure.
- Although lacrimation was observed clinically among the rats exposed to 40 ppm during and shortly following exposure, no histopathological correlate of the response was evident. No damage to the cornea or conjunctiva was observed.
- Rats are obligate nasal breathers. Accordingly, the observed effects of H_2S on the nasal epithelium might be expected to be more severe in this specie compared to other species. Differences in ventilation rates and in the morphological architecture of the nasal passages between species also could affect outcome.
- The study findings and conclusions were based on the use of male rats only.

Confidence index:

The study was reasonably well designed, conducted and reported. On balance, the study did satisfy the recommendations contained in testing guidelines for acute toxicity studies. Some weaknesses were noted, but they were relatively minor and did not significantly detract from the level of confidence that could be assigned to the study findings and conclusions. The approach taken was objective and the conclusions reached were supported by the results.

Review – Confidence index ranking:

Moderate to high

Document Review

Author:	Barilyak, IR; Vasil'eva, IA	**Internal ID:**	497
Title:	Antimitotic and cytogenetic activity of carbon bisulfide and hydrogen sulfide in small concentrations.		
Year:	1974		
Abstract:	*Not available*		
Objective:	To investigate the effects of exposure to small concentrations of carbon bisulfide and hydrogen sulfide on both the mitotic activity of embryonic cells and on the chromosome apparatus of bone marrow cells in mature rats. [NB: Four separate experiments were conducted involving exposure of male and/or female rats prior to mating, during mating and/or during gestation. With the exception of the third experiment (*i.e.*, Series III), each study involved prolonged exposure (70-90 days) of the parental animals. Consequently, only the third experiment fit the inclusion criteria for the present review]		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm; 7.14ppm (presumed)	Repeated/ Unknown number of hours per day from the first to twentieth day of pregnancy.	Rat	"*unpedigreed*"	3-4 months	Female	Not specified.	Not specified.	7.14ppm	NA

Observations:

- Mitotic activity of hepatic embryonic tissues: 16.5% decrease relative to controls
- Mitotic activity of renal embryonic tissues: 0.7% decrease
- Changes in mitotic activity did not achieve statistical significance.

*Statistically significant

Author's Conclusions:

- *"It must be noted that the antimitotic effect of carbon bisulfide and hydrogen sulfide was manifested only after prolonged exposure. Exposure of the animals throughout pregnancy did not have this effect: the mitotic index in the hepatocytes and renal cells of the embryos was identical to that in the control animals."*

Review – Assessment:

A. Test animals:	- The number of animals used was not defined. +/- Minimal information was provided pertaining to test animal characteristics. The pre-test health status and weight variation of animals was lacking. + The source of the test animals was indicated. - Test animals were "*unpedigreed*". - Unclear whether all animals used were of 3-4months of age or if this only described those rats used in the first experimental series.
B. Exposure conditions:	- The source of H_2S was not indicated.

	- The number of hours which animals were exposed per day was not defined. - Unclear whether a whole body exposure chamber was used. - Whether oxygen content, temperature, pressure, humidity, photoperiod and H_2S concentrations were monitored throughout exposure was not reported. - The exposure concentration was unclear. It was stated that rats *"were exposed to small concentrations of carbon bisulfide and hydrogen sulfide (10mg/m³)"* in the first series of experiments. Whether this concentration pertains to both CS_2 and H_2S and if the exposure concentration was identical in all four experiments is unknown. - Animals were exposed to both H_2S and CS_2 simultaneously.
C. Housing/Feeding:	- Details concerning animals housing (*i.e.*, temperature, humidity, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type, dimensions, etc.) was not provided. - Bedding and nesting material was not specified. - The type and source of feed supplied was not reported. The feeding schedule was omitted. - Details concerning the water supply were lacking.
D. Equipment:	- No details pertaining to the exposure system were provided. - Monitoring equipment was not described.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. - Absence of information pertaining to the randomization of test animals and assignment to test groups. +/- A control group was reportedly included, but details concerning handling and disposition were completely lacking. - Conventional measures of developmental toxicity (*e.g.*, maternal body weight, maternal feed intake, length of gestation, pre- and post-implantation losses, litter parameters, fetal parameters, *etc.*) were not included. - A single exposure concentration was tested, thereby, precluding any examination of dose-responsiveness. - Unclear whether technicians and handlers were blinded to exposure conditions. - It was stated that *"20-31 thousand cells was evaluated in each case"* but from how many animals these cells were extracted from was not defined. - Study pre-dated Good Laboratory Practice (GLP) conditions.
F. Data collection:	- Individual data were not provided for each test animal, thereby limiting the independent assessment of the findings.
G. Data analysis:	- Data was analyzed by *"Variation statistics"* . The type of analyses this term includes was not stated. - Both statistical significance of results and confidence intervals were not reported.
H. Interpretations:	- The authors' conclusions from various experimental series were grouped and therefore differentiating sub-acute effects from those observed following chronic exposure was impossible. - Rats were exposed to both carbon disulfide and hydrogen sulphide simultaneously and no test group was exposed to a single chemical substance. As a result, symptoms cannot be attributed to hydrogen sulfide alone. - Potential confounding variables were not limited by either experimental design or statistical analyses.

Review - Summary:

Discussion of findings:

The exposure of unpedigreed white rats to low levels of carbon bisulfide and hydrogen sulfide simultaneously from the first to twentieth day of pregnancy, resulted in no significant change in the mitotic activity of either hepatic or renal embryonic tissue.

Interpretation of the significance of experimental findings was limited by a general lack of information and ambiguity with respect to most aspects of study design and conduct. Interpretation was rendered very difficult by:

- Simultaneous exposure to both carbon bisulfide and hydrogen sulfide. Even if effects on mitotic activity had been discovered, it would be impossible to assign cause to H_2S alone.
- Uncertainty with respect to the exposure concentration used. Confusion surrounds not only the target concentration of H_2S, but also the actual concentration that might have been received by the dams given the complete lack of information respecting exposure conditions.
- General lack of detail concerning the test animals, including lack of information respecting group sizes, housing conditions, and husbandry.
- Lack of detail concerning selection and processing of maternal and fetal tissues for analyses of mitotic index.

Confidence index:

Confidence in the study findings and conclusions was seriously undermined by numerous weaknesses in design, conduct and reporting. The weaknesses included:

- Lack of information pertaining to number of test animals, and animal characteristics.
- Failure to include test groups in which animals were exposed to a single chemical (*i.e.*, either CS_2 or H_2S).
- Ambiguity with respect to exposure concentrations.
- Lack of detail with respect to exposure conditions.
- Failure to describe exposure or monitoring equipment.
- Absence of masking procedures and randomization.
- Omission of conventional measures of toxicity.
- Unknown statistical methods.

Review – Confidence index ranking:

Low

Document Review

Author:	Hayden, LJ; Goeden, H; Roth, SH	**Internal ID:**	564
Title:	Exposure to low levels of hydrogen sulfide effects circulating glucose in maternal rats.		
Year:	1990		
Abstract:	*"Although the lethal effect of hydrogen sulfide (H_2S) has long been known, the results of exposure to low levels of H_2S have not been well documented. Rat dams and pups were exposed to low levels of H_2S ($\leq$ 75ppm) from d 1 of gestation until d 21 postpartum and analyzed for changes in circulating enzymatic activity and metabolites. Blood glucose was significantly elevated in maternal blood on d 21 postpartum at all exposure levels. This increase in glucose was accompanied by a possible decrease in serum triglyceride in the pups and in the dams on d 21 postpartum. There was no evidence of alterations in serum alkaline phosphatase, lactate dehydrogenase, or serum glutamate oxaloacetate transaminase."*		
Objective:	To determine whether low levels of H_2S ($\leq$75ppm) alter maternal, fetal, or neonatal growth and development using various metabolic and enzymatic markers in the rat. (The markers included serum glucose, serum triglycerides, serum cholesterol, lactate dehydrogenase, alkaline phosphatase, and glutamate oxaloacetate transaminase).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0ppm: 20ppm; 50ppm; 75ppm	Repeated/ 7 hours a day from day 1 of gestation until day 21 postpartum. (It would appear that exposures were not performed concurrently).	Rat	Sprague-Dawley	Dams were 80 to 90 days of age at time of mating	Both	20 gravid females were reportedly placed in two groups. Each group was culled to 6 litters with 12 pups in each litter	Not specified.	NA	20ppm

Observations:

- Serum glucose levels (relative to control values):
  - Maternal (Day 21 post-partum):
    - 20ppm: 37% increase*
    - 50ppm: 44% increase*
    - 75ppm: 50% increase*
  - Pup:
    - 20ppm:
      - day 7: 5% decrease
      - day 14: 3% decrease
      - day 21: 1% increase
    - 50ppm:
      - day 7: 13% decrease
      - day 14: 2% decrease
      - day 21: No change
    - 75ppm:
      - day 7: 3% increase

        - day 14: 7% increase
        - day 21: 3% increase

- Serum triglyceride levels:
    - Maternal:
        - 20ppm: 16% decrease
        - 50ppm: 25% decrease*
    - Pup:
        - 20ppm:
            - day 7: 2% decrease
            - day 14: 11% decrease
            - day 21: 8% increase
        - 50ppm:
            - day 7: 19% decrease
            - day 14: 19% decrease*
            - day 21: 20% decrease

- Serum cholesterol levels:
    - Maternal:
        - 20ppm: 23% decrease
        - 50ppm: 14% increase
    - Pup:
        - 20ppm:
            - day 7: 8% decrease
            - day 14: 1% increase
            - day 21: 6% decrease
        - 50ppm:
            - day 7: No change
            - day 14: 13% increase
            - day 21: 26% increase*

- Serum alkaline phosphatase activity: No significant changes in either dams or pups at any exposure concentration.

- Serum lactate dehydrogenase activity: No significant changes in either dams or pups at any exposure concentration.

- Serum glutamic oxaloacetic transaminase activity: No significant changes in either dams or pups at any exposure concentration.

- Serum protein: No significant changes in either dams or pups at any exposure concentration.

- For serum protein and enzyme activities, differences from control values were routinely less than 10%, and no dose-related patterns were evident.

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*Statistically significant

Author's Conclusions:

- *"…exposure of pups to…H_2S did not significantly alter the circulating levels of glucose. However, dams from these same litters had glucose levels that were approximately 50% greater in the animals at all exposure levels" "…elevation in maternal glucose after long-term exposure to low levels of H_2S could be the result of either a reduction in uptake and metabolism of glucose or the overproduction of glucose by adult hepatic tissue. The reason for the difference in glycemic response between adult and neonate rats was not apparent…This perturbation of glucose metabolism could be secondary to a decrease in metabolism…" "…further studies will be required to determine if serum glucose levels are elevated because of disruption of essential disulfides involved in hormonal control of metabolism or because of a perturbation of metabolism at a postreceptor site and to determine if the developing rat will develop hyperglycemia with maturity" "A growing body of evidence supports the hypothesis that chronically elevated glucose levels can produce retinopathy, neuropathy, and nephropathy and can increase the severity of cardiac and peripheral atherosclerosis."*

- *"Triglyceride concentrations were reduced in the pups and dams in an apparent dose-dependent manner…" "Serum triglyceride and cholesterol levels were approximately threefold higher in the pups than in maternal circulation." "The suppression of serum triglyceride levels also suggested that there was decreased use of carbohydrates for de novo synthesis of circulating triglycerides in the liver, the major site for synthesis of circulating triglycerides."*

- *"Serum protein, LDH, SGOT, and alkaline phosphatase activities were determined…no differences between the mean serum activities from exposed animals and activities from the control animals." "Since neither serum LDH nor SGOT activities were altered in these animals, no tissue damage producing enzyme leakage was apparent…" "…in the present study neither blood, brain, nor liver alkaline phosphatase activity was decreased."*

Review – Assessment:

A. Test animals:	- The number of animals used in the trial was unclear. The authors state that *"Twenty gravid females were placed in two matched groups.."* but whether this was per exposure group was not specified. - The number of litters examined was inconsistently reported. The Methods section states that *"each group was culled to 6 litters with 12 pups in each litter at parturition"*. However, the Results section shows that up to 18 litters in any given group were examined. It is suspected that exposures may have been performed in replicate, with 6 litters examined in each trial, but this was not clearly stated in the report. - The basis for culling to 6 litters on Day 1 post-partum was not indicated. Guideline recommendations generally allow for culling on pups within any given litter, usually on Day 4 post-partum. No guideline recommendations pertain to the culling of litters, and the practice is generally discouraged owing to loss of experimental data. - Use of only 6 litters per exposure group fails to meet guideline recommendations. Guidelines generally recommended that 20 litters per dose group be followed and examined. +/- The principle characteristics of test animals (*e.g.*, specie, breed, age, sex) were adequately reported, however, information pertaining to weight variation and pre-test health status were not addressed. - Source of test animals was not reported.
B. Exposure conditions:	+ Source of H_2S gas was noted. - The exposure duration was unclear as it was quoted differently within the article. Within the Methods section, exposures were stated to be 7 hours/day, whereas in the Discussion section, the exposure time is listed as 6 hours/day. - A whole body exposure chamber was employed. +/- Chamber conditions were monitored for airflow, pressure, temperature, humidity, and H_2S concentration. For certain parameters, it could not be determined as to whether or not the chamber conditions complied with guideline recommendations.

	+ Oxygen concentrations within the chamber were maintained at 20-21%, consistent with guideline recommendations. + Airflow through the chamber was kept at a flow rate of 20 changes/hr. + Equilibration of H_2S concentration within the exposure chamber reportedly was achieved within 10 minutes. + Actual concentration of H_2S in the chamber was reportedly maintained within 5% of the desired concentration at all times. + The concentration of H_2S was measured at the level of the animal's breathing zone.
C. Housing/Feeding:	+ The material from which cages were constructed was noted, but the dimensions of these cages was not specified. + Animals were individually housed, except during mating. +/- Feed and water were provided *ad libitum*, except during exposure. The type of feed was not reported. Source of feed and water supply were not given. +/- The environmental conditions under which the animals were housed (*e.g.*, temperature, humidity, etc) were not defined except that a 12-hour photoperiod was maintained. - Bedding type was not specified.
D. Equipment:	+ The material from which the exposure chambers were constructed (acrylic) and dimensions were noted. + The total animal volume in chambers did not exceed 5% of the total volume of the chamber, consistent with guideline recommendations. + The monitoring equipment used to assess concentration and airflow within the chamber was adequately described. +/- Additional details concerning the exposure chamber and gas delivery system were available in a companion paper (Laskin and Drew, 1970).
E. Procedural:	+/- Animals were acclimatized, but the period of acclimitization was half of that generally recommended. - It was unclear if rats were randomly assigned to exposure groups. - It was unclear if the culling of litters was performed randomly. + A control group of animals was included and the controls were maintained under identical conditions except for exposure. +/- Exposed animals were continuously observed, but details concerning clinical observations were not reported.. - It is unclear whether technicians and handlers were blinded to exposure conditions. +/- Serum was collected at a specified time for analysis of serum triglyceride and cholesterol levels, so the interval between last meal and analysis was comparable. However, it is not clear whether or not the prandial state of the dams also was controlled for the analysis of serum glucose, protein and enzyme levels. - The culling of litters on Day1 post-partum is generally not recommended. Instead, most guidelines recommend maintenance of all litters, and culling of pups on Day 4 post-partum to avoid confounding of results due to variations in litter size during the post-natal period. - The culling of litters such that each retained litter contained 12 pups is not conventional practice. Typically, litters are culled to 8 pups to avoid undue stress on the dam post-partum and to reduce inter-litter variability, both of which can confound interpretation of test results. - Handling of the animals was not clearly indicated. It is not known whether the animals were housed in the exposure chamber throughout the gestation and post-partum periods or whether they were transferred in and out of the chamber on a daily basis for the 6 to 7-hour exposure period. - The overall performance of the study was not clearly indicated in terms of the assignment of animals to exposure groups and the number of replicate experiments, if any, that were completed. The performance of the study is confused by the inconsistent reporting of the number of litters that were followed and examined. - It is not clear why analyses of serum triglycerides and cholesterol were confined only to 20ppm and 50ppm, and did not extend to 75ppm. This is especially confusing since the basis for measuring the triglycerides and cholesterol was *"because of the increase in circulating glucose at 75 ppm"*.

	- No indication that the study was conducted under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- Conventional measures of toxicity were not performed (*i.e.*, clinical observations, body weights, feed consumption, clinical pathology, organ weights, gross pathology and/or histopathology). - Individual animal data were not supplied.
G. Data analysis:	+/- Statistical analysis of the data was carried out using analysis of variance and Duncan's multiple range test or a Student-Newman-Keuls test. - Use of the litter as the only unit of comparison is generally not recommended, due to possible "skewing" of results. Analysis on both a fetus and litter basis should have been performed. - The data suggest that statistical comparisons were based on litter numbers which differed between exposure groups, ranging from 6 to 24.
H. Interpretations:	+ The original objective of this study was adequately addressed by the experimental procedure. - Use of the litter only as the basis of comparison between exposure groups limits the extent to which significance can be assigned to the findings. - The apparent reported decrease in serum triglyceride levels in the pups was not obvious. The change was not dose-related, nor consistent across the various sampling times.

Review - Summary:

Discussion of findings:

Exposure of gravid female rats to graded exposure concentrations of H_2S from Day 1 of gestation to Day 21 post-partum resulted in a dose-related increase in circulating glucose levels and a corresponding decrease in serum triglyceride levels. Serum cholesterol levels were unaffected. Circulating levels of glucose, triglycerides and cholesterol in the pups were largely unchanged. Differences from control pup values were not consistent across the various sampling times, nor were the changes uniformly dose-related. No changes in serum protein levels or the activities of alkaline phosphatase, lactate dehydrogenase or glutamate oxaloacetate transaminase were evident in either the dams or pups at any exposure concentration or sampling time. The latter findings suggest a lack of any tissue injury as a result of exposure.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The changes in maternal serum glucose and triglyceride levels achieved statistical significance in most instances, with differences evident even at the lowest exposure concentration of 20 ppm. The significance of the reduction in circulating triglycerides is enhanced by the fact that the prandial state of the dam was stabilized by control of feeding at the time of sampling. It is not known whether the same control applied to the glucose measurements, thereby rendering interpretation of these findings difficult.
- Interpretation of the meaning and significance of the study findings could have been improved through better description of the protocol followed for the treatment of the animals. Inconsistencies in the reporting of the number of dams and litters selected for examination detracts from the understanding of the study protocol and erodes the level of confidence assigned to the findings and conclusions. It would appear that a series of treatments, involving different exposure concentrations, was performed and the results were then combined. This confounds the interpretation of the findings.
- Interpretation of the significance of the findings also would have benefited from comparison of the results on both a fetus and litter basis. Testing guidelines for developmental toxicity generally discourage use of the litter as the only unit of comparison because variations in responses between littermates may be lost through this type of analysis. The guidelines generally recommend provision of data on both a fetus and litter basis to allow for better understanding and assessment of effects.
- The toxicological significance of the changes in circulating maternal glucose and triglyceride levels is not well established. The responses were not accompanied by corresponding changes in the pups, nor was there any evidence of cellular or tissue injury among the dams as a result of treatment. Regrettably, details concerning clinical signs were not reported. Accordingly, it was not possible to correlate the changes in serum glucose and

triglycerides with clinical signs that might accompany hyperglycemia and/or hypotriglyceridemia. Moreover, there was no evidence to suggest that the changes in maternal parameters affected the fetuses.

- Although the assessment of the effects of H2S exposures on fetal development is of importance, the meaning and significance of the findings from the present study remain largely unknown. As the authors suggest, further study is needed to better understand the observed changes in maternal glucose homeostasis. It is also important to consider the nature of the exposure regimen followed in the study in assessing the relevance of the findings to the human condition. The dams were exposed to up to 75 ppm of H2S for 6 to7 hours per day for up to 42 days, including the entire period of gestation and the entire length of the weaning period. The equivalent exposure time in humans would extend over several months.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Lack of detail with respect to the handling of the test animals.
- Lack of detail with respect to assignment of animals to exposure groups and selection of litters for examination.
- Departure from recommended testing guidelines, especially with respect to culling of litters.
- Inconsistent reporting of exposure times.
- Inconsistent reporting of the number of litters examined.
- Use of the litter only as the unit of comparison.
- Use of too few litters for certain exposure concentrations to permit meaningful comparison.
- Failure to include routine measures of toxicity, including clinical observations.

Review – Confidence index ranking:

Moderate to low

Document Review

Author:	Kosmider, S; Rogala, E; Dwornicki, J; Szulik, Z	**Internal ID:**	595
Title:	The influence of Vitaral on some mineral and enzyme disturbances in subacute poisoning with hydrogen sulfide		
Year:	1971		
Abstract:	*"Investigations have been made with rabbits to indicate some disturbances in the living organism brought by hydrogen sulfide (H_2S) about poisoning and to see if administration of some trace metals present in Vitaral has any effect upon these disturbances.* *In the course of the investigation protein, enzymatic and mineral disturbances as well as acid-base balance disorders were found. The disturbances did not occur when the exposed rabbits received Vitaral with their food.* *The investigations were made in order to determine how H_2S exerts its poisonous action upon the brain kidney, heart and liver. The H_2S mechanism is based on its ability to bind with alkali metals which, among others, play an important role as activators of some of the enzymes. As a result of the binding, the enzymes are deprived of their metal activators.* *This accounts for the inhibition of the examined enzymes. Vitaral administered to the poisoned animals substituted the lacking trace metals which was followed by reactivation of the disturbed enzymes."*		
Objective:	To investigate protein, enzyme, acid/base and mineral disturbances following H_2S exposure in rabbits. (Note that the study involved treatment of rabbits with H_2S alone or in combination with Vitaral, a mineral supplement. The major focus of the present review was directed at the animals treated with H_2S alone. In addition, the study included the examination of human serum enzymes exposed to H_2S vapours under *in vitro* conditions. The findings from these *in vitro* studies were not considered in the current assessment).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.1mg/L = 71ppm	Repeated/ 1 hr/day for 14 days	Rabbit	Not specified. (Reported only to be *"mixed"*)	One-year	Both	70 divided into four groups. (a) Control (20 animals) (b) Exposed (20 animals) (c) Exposed with Vitaral in food (20 animals) (d) Positive control (10 animals) (*i.e.*, exposed to Vitaral alone)	Unknown	NA	71ppm

Observations:

- Clinical symptoms:
  - Behavior: Initial excitement, changing to drowsiness.
  - Respiratory: Accelerated breathing
  - Cardiovascular: Accelerated pulse rate
  - Ocular: "*congestion*" of the conjunctiva

- Serum protein levels:
  - [Albumin]: Decrease
  - [Globulin]: Increase

- Blood mineral levels:
  - Calcium: 2% increase
  - Magnesium: 21% decrease*
  - Iron: 16% decrease*
  - Copper: 31% decrease*

- Acid/Base balance:
  - pH: 1% decrease*
  - Partial CO_2 pressure (mm Hg): 8% decrease
  - $C0_2$ content (volume %): 23% decrease*
  - Buffer base (Meq/l): 11% decrease*
  - Plasma bicarbonate (Meq/l): 19% decrease*

- Thymol turbidity: 73% increase*

- Liver enzymes
  - Glutamic-oxaloacetic activity: 80% increase*
  - Glutamic-pyruvic transaminase activity: 4% decrease

- Alkaline phosphatase activity:
  - Serum: 26% decrease*
  - Brain: 21% decrease
  - Heart: 27% decrease*

- Caeruloplasmin activity:
  - Serum: 36% decrease*
  - Brain: 24% decrease*
  - Heart: 22% decrease

- Histochemical:
  - Liver:
    - Succinyl dehydrogenase: Decrease
    - Acid phosphatase: Increase
    - ATP-ase: Decrease in some exposed rabbits
    - Alkaline phosphatase: Decrease
  - Kidney:
    - Cortical succinyl dehydrogenase: Decrease
    - Acid phosphatase , Henle loop cells: Increase
    - ATP-ase :
      - Cortical: Decrease
      - Medullary: Increase

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*Statistically significant

Author's Conclusions:

- *"Poisoning with H_2S produces protein and mineral disturbances..."*
- *"H_2S poisoning produces damage to the liver ..."*
- *"H_2S disturbs brain, liver and kidney metabolism ..."*
- *"H_2S leads to a decrease in the total serum protein content, mainly globulin."*
- *"The results indicate that H_2S produced mineral disturbances due to its binding with metal ions to form sulfides. This accounted for the inhibition of some of the enzymes in the liver and kidney."*
- *"In consequence of mineral and enzymatic disturbances, the acid-base balance is effected..."*
- *"Poisoning caused acid-base balance disorders since a decrease of pH, total carbon dioxide content and carbon dioxide pressure were observed."*
- *"The H_2S poisoning produces damage to the liver"*, as indicated by both the *"increase in the value of thymol turbidity test"* and *"increase in glutamic-oxaolacetic and glutamic-pyruvic transaminase activity."*
- *"The data clearly showed that H_2S damaged the liver and disturbed kidney metabolism."*

Review – Assessment:

A. Test animals:	+ Adequate numbers of test animals were allocated to each exposure group. - The experiment was conducted on rabbits of mixed breed and sex. The distribution of sexes across exposure groups was not indicated. Actual breed was not specified. + Exposed animals were adults, in accordance with guideline recommendations. - The breeding facility from which the animals were obtained was not noted. - It was not noted whether a pre-test health assessment had been completed. + The 18% weight variation of test animals was below the maximum guideline recommendation.
B. Exposure conditions:	+/- A whole body exposure chamber was used. - The source of H_2S was not given. - It is unclear whether animals were placed in the exposure chamber before or after the equilibration period. In the latter case, a short equilibration period is desirable. + The exposure period was precisely defined. - Airflow, temperature and humidity were not monitored. - No justification was provided for the dosing regimen.
C. Housing/Feeding:	- No information pertaining to housing conditions was provided (*e.g.*, temperature, humidity and photoperiod). - Information respecting caging was not given. - Bedding material was not specified. - Feeding schedule was not defined. - Feeding restrictions imposed during exposure were not indicated. + Test animals were fed a *"standard"* diet before and during the experiment, but the feed was not commercially prepared Instead, the diet consisted of *"fresh vegetables, oats and hay"*. - Details concerning water supply were lacking.
D. Equipment:	- Information pertaining to the exposure chamber and gas delivery system was lacking. The exposure chamber was simply described as a *"specially constructed chamber"*. - Gas was evidently delivered by a *"Kipp generator"*.

	- No details were given concerning flow rates, pressures, temperature, humidity *etc.* - A Draeger apparatus was reportedly employed to measure the H_2S concentration within the exposure chamber.
E. Procedural:	- No acclimation period or pre-test conditions (*i.e.,* quarantine, disease treatment, etc) were specified. - It was unclear whether test animals were randomly assigned to exposure groups. - Whether control animals also were placed in the exposure chamber, to account for the effect of stress, is not apparent. - The period of time required to bleed the animals to death was not stated. If prolonged this may have accounted for some of the hypoxic damage observed. + Same serum was used in all five parts of analysis. - Whether technicians and handlers were blinded to exposure conditions was not stated. - Conventional measures of toxicity, including body weight, routine clinical pathology and gross and histopathology were not performed. - Clinical observations were limited. Duration, frequency and severity were not specified, nor was an indication of the number of animals affected given. - A single exposure concentration only was tested. Testing guidelines generally recommend using of graded exposure levels to permit determination of dose-responsiveness. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	- If technicians conducting the assays were blinded to the exposure levels this was not noted. +/- Although individual animal data were not provided, the study findings were summarized in extensive data tables and photomicrographs.
G. Data analysis:	- The statistical methods employed were not reported. - No confidence intervals were provided.
H. Interpretations:	- Bias might have been introduced due to several deficiencies in the experimental design (e.g., absence of blinding, lack of and randomization, etc.) - Lack of detail concerning exposure conditions renders interpretation of the study findings difficult. - Inconsistent reporting of results. Serum globulin levels were reportedly both increased and decreased following treatment. - Use of only a single exposure concentration precludes assessment of dose-responsiveness of reported changes in mineral levels, acid/base balance and enzyme activities.

Review - Summary:

Discussion of findings:

Rabbits exposed to 71ppm H_2S for 1hr/day for 14 days exhibited changes in serum and/or tissue protein, mineral and enzyme levels. Certain of the changes were reportedly indicative of liver and kidney damage. Conventional measures of toxicity were largely lacking, but clinical observations revealed rapid respiration, elevated heart rate, and initial excitement at the time of exposure. Eyes reportedly developed *"congestion"* of the conjunctiva.

Interpretation of the significance of the study findings should take into consideration the following:

- The reported *"congestion"* of the conjunctiva following exposure to H_2S was poorly defined (*i.e.,* lack of description, lack of detail concerning number of rabbits affected, lack of indication of severity of condition). Regrettably, the nature of the "congestion" was not elucidated through *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination of the eyes.
- Although the authors concluded that damage to the liver and disturbance of kidney function were produced by exposure to H_2S, this conclusion was based entirely on changes in the activities of certain enzymes, notably SGOT, SGPT, alkaline phosphatase and ATP-ase. Unfortunately, no histopathological correlates of the changes were determined owing to limitations in the study design (*i.e.,* histopathological examination of tissues was

not performed).

- The dose-responsiveness of the reported changes could not be determined owing to the use of a single exposure concentration only. Confidence in the study findings could have been improved through use of graded exposure levels, provided meaningful dose-response patterns emerged.
- The clinical and toxicological significance of the observed changes are not easily assessed. Apart from the lack of conventional measures of toxicity that might have aided interpretation through determination of clinical and/or pathological correlates, the study also provided no point of reference for the various shifts in protein, mineral and enzyme levels that might assist in establishing clinical relevance (*i.e.*, the shifts may have resided within the range typically accommodated by normal homeostatic mechanisms).
- The reversibility of the reported shifts in protein, mineral and enzyme levels is unknown. The study might have been improved by the addition of a recovery phase.
- The activities of the majority of the enzymes examined were based on histochemical analyses (*i.e.*, alkaline phosphatase, succinyl dehydrogenase, acid phosphatase, ATP-ase), rather than on conventional assay methods which are more specific, more precise, and more reproducible.
- The relevance of the findings to the human condition is confounded by the lack of detail concerning the pre-test health status of the test animals as well as the distribution of sexes within the treatment groups.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Inadequate description of the exposure chamber and gas delivery system. Description was limited to the statement that rabbits were exposed in a "*specially constructed chamber*" into which H_2S was introduced by "*a Kipp generator*".
- Inadequate description of exposure conditions.
- Use of a single exposure concentration only, thereby precluding assessment of the dose-responsiveness of the observed changes in protein, mineral and enzymatic levels.
- Lack of detail to confirm that the target exposure concentration was attained. The report states only that "*the H_2S concentration was measured by a Draeger apparatus*". The frequency of measurement was not given, nor were the differences between targeted and measured concentrations provided. The reliability of the method also is questionable.
- Complete lack of detail concerning animal housing and husbandry.
- Lack of detail concerning randomization procedures (*e.g.*, assignment of animals to treatment groups, bleeding schedule).
- Failure to include most routine measures of toxicity as part of the overall study design, including body weights, clinical pathology, gross pathology, organ weights and histopathology, which could have assisted in the interpretation of the significance of the findings.

Review – Confidence index ranking:

Low

Document Review

Author:	Roth, SH; Hannah, RS	**Internal ID:**	630
Title:	The neurophysiological effects of low concentrations of hydrogen sulphide on the developing and mature nervous systems		
Year:	1989		
Abstract:	*"It is well established that the environmental pollutant, hydrogen sulphide (H_2S), can be extremely toxic to both animals and humans at high concentrations; however, there is little information regarding the effects of long term exposure to low levels. H_2S is a major component o sour gas and a by-product of more than 70 industrial operations. It has been referred to as a broad spectrum toxicant that can elicit a wide variety of biological responses. Prolonged exposure to low concentrations may be a form of "chronic poisoning". Numerous neurophysiological symptoms have been associated with long term exposure to low levels, such as nervousness, poor memory, headache, sleep disturbances, insomnia, drowsiness, fatigue, weakness of extremities, disturbed equilibrium, vertigo, convulsions, anxiety, agitation, and delirium. Exposure to high concentrations can produce unconsciousness, coma, paralysis of respiratory and cardiovascular centers leading to death. These effects strongly suggest the central nervous system (CNS) is the major target organ for H_2S toxicity. Studies in our laboratory using in vitro neuronal model preparations have confirmed that low concentrations of H_2S can produce both a concentration and time-dependent alteration of neuronal function. Developing and/or immature organisms are particularly susceptible to the adverse effects of most toxicants. We have studies the effects of low concentrations of H_2S on the developing organism and have observed that the dominant effects of H_2S appear to occur in the developing CNS. Exposure to H_2S produced a significant increase in the density of Purkinje cells and decrease in the granule/Purkinje cell ratio in the cerebellum of the developing rat. There was also a significant alteration in both the dendritic architecture and overall growth process. Levels of putative amino neurotransmitters (aspartate, GABA, glutamate, taurine but not glycine) were altered in the exposed developing rat brain. Although the effects of low concentrations of H_2S on neuronal tissue may be subtle, the results of our studies may explain the many behavioral responses reported and suggest that structural and functional abnormalities may occur during development."*		
Objective:	This document was a review paper in which four separate experiments were summarized, with a primary focus on the effects of H_2S on the developing nervous system. Experiment (a) investigated H_2S alterations on neuronal function using an *in vitro* model involving treatment of sensory neurons isolated from crayfish with sodium sulfide, sodium bisulfide, and gaseous H_2S. Experiment (b) explored the effect of H_2S on the density of cerebellum cell types in developing rats. Experiment (c) explored the impact of H_2S on the growth and development of Purkinje cells (previously reviewed as part of Study I.D. 11 – Hannah and Roth, 1991). Experiment (d) explored variations in amino acid neurotransmitter levels in the developing rat nervous system (previously reviewed as part of Study I.D. 180 – Hannah *et al.*, 1989). Review was limited to Experiment (b). This experiment was described previously as a *"preliminary study"* (Hannah *et al.*, 1989).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 ppm; 75ppm (whole body)	Repeated/ 7-hours per day from day 5 post-coitus until day 21 postnatal	Rat	Sprague-Dawley	Not specified	Pregnant females; Sex of pups was not specified.	Number of dams not specified; 32 pups (16 treated; 16 control)	Timed-pregnant	NA	75 ppm

Observations:

- Mean Purkinje cell number:
  - Postnatal day 7: ~38% higher than control
  - Postnatal day 14: ~35% higher than control

- Mean granule cell number:
  - Postnatal day 7: 1% higher than control
  - Postnatal day 14: 5% lower than control

*Statistically significant

Author's Conclusions:

- *"Exposure to 75ppm H_2S resulted in a 20% increase in the density of surviving Purkinje cells....whereas there was no significant change in the mean number of granule cells"*
- *"Exposure to H_2S produced a significant increase in the density of Purkinje cells and decrease in the granule/Purkinje cell ratio in the cerebellum of the developing rat".*
- *"The complexity of the CNS in both structure and function often yields results that are difficult to interpret".*
- *"... the effects of low dose, chronic exposure to H_2S may result in perturbations of the normal growth process of the developing CNS ultimately leading to abnormal function".*
- *"The observation that H_2S can produce morphological changes in the cerebellum suggests that many other neuronal populations may be at risk".*

Review – Assessment:

A. Test animals:	- Number of dams on test was not indicated, nor was pre-test health status, age, weight variation and source. - Number of litters from which pups were selected for examination was not specified. - The number of pups examined was reported (*i.e.*, 8 per exposure group at each sampling time), but the number of litters from which the pups were secured was not specified. Given that guideline recommendations for developmental toxicity studies generally require the use of 20 dams per exposure group and examination of the corresponding litters that typically consist of 2 to 12 pups (*i.e.*, total of 40 to 240 pups per group), the number of pups examined in the study was judged to be limited.
B. Exposure conditions:	- The source of the H_2S gas was not provided. + The duration of exposure was precisely defined. +/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. +/- H_2S concentration was reportedly continuously monitored throughout exposure; however, differences between the measured concentrations and target concentration were not provided. - The temperature, humidity and pressure maintained within the exposure chamber were not specified.
C. Housing/Feeding:	- No details concerning animal housing or husbandry were given.
D. Equipment:	+/- Only a limited description of the exposure chamber and gas delivery system was provided; however, further details were reported by Laskin and Drew (1970). + The equipment used to monitor the gas concentration and airflow rate was specified.
E. Procedural:	- No acclimatization period was reported. - No description of pre-test conditions was provided.

	+/- Pups were randomly chosen for euthanization, but the method of randomization (*e.g.*, table of random numbers, computer generated, etc.) was not reported. - Only a single exposure concentration (*i.e.*, 75 ppm) was used. Testing guidelines generally require the use of graded exposure concentrations, with 3 dose levels and a control group recommended. + A control group of animals was included and the controls were maintained in the same conditions without H_2S exposure, consistent with guideline recommendations. +/- Unclear whether technicians and handlers were blinded to exposure conditions. - No indication that study was conducted under Good Laboratory Practice (GLP) conditions. - Conventional measures of developmental toxicity (*e.g.*, clinical observations, body weights, litter parameters) were not included as part of the study design. - Details concerning methodology used to quantify number of Purkinje and granule cells were not available.
F. Data collection:	- Individual data were not provided for each animal, thereby limiting the independent assessment of results. - Findings were presented in graphical form only. No absolute numerical data were reported.
G. Data analysis:	- The nature of statistical analysis conducted was not specified.
H. Interpretations:	- The reported possible association between the changes in the number of Purkinje cells in the cerebellum and abnormal neuronal function represents a hypothesis only, without support of empirical data. - Limitations in study design (*e.g.*, use of limited numbers of pups, use of only a single exposure concentration) restrict interpretation of the significance of the study findings. The results should be considered preliminary in nature, with further study needed to confirm the findings and to determine their significance.

Review - Summary:

Discussion of findings:

In utero and post-natal exposure of rat pups to 75 ppm H_2S for 7 hours per day from Day 5 post-coitus to Day 21 post-partum reportedly produced morphological changes in the cerebellum, characterized by increased numbers of Purkinje cells compared to control pups. The number of granule cells was unchanged following treatment. The authors postulated that the morphological changes might predispose the animals to abnormal neurological function, albeit it was admitted that the interpretation of the findings was *"very difficult"* owing to the complexity of the developing nervous system.

Limitations in study design, conduct and reporting limit the significance that can be assigned to the study findings and conclusions. For example, the use of a single exposure concentration only precludes any assessment of the dose-responsiveness of the observed changes. Use of graded exposure concentrations would have allowed for more meaningful interpretation of the results by possibly strengthening the association between the reported changes and exposure to H_2S. In addition, only a restricted number of pups were examined at each sampling time, *and* details concerning pup selection were completely lacking, thereby further adding to the difficulty of interpreting the significance and relevance of the findings. In general, the paper suffered from a lack of description surrounding test methods and study conduct that undermines the level of confidence that can be assigned to the findings and conclusions. More complete descriptions would have added considerably to the ease of interpreting the results. Regrettably, the study did not include routine measures of toxicity, such as clinical observations of the test animals, which might have allowed for better assessment of the possible association between the observed changes in Purkinje cell numbers and neurobehavioral abnormalities, as postulated by the authors. In the absence of such information, the association remains unsubstantiated and must be considered tenuous. It is also difficult to interpret the significance of the reported changes given current understanding of the 'functional reserve' of the central nervous system. The overall impact of the observed increase in Purkinje cell density on the functional integrity of the CNS is unknown.

Any assessment of the relevance of the observed changes to the human condition should consider the nature of the exposure regimen employed, involving repeated daily exposure to H_2S for much of the gestation period and pre-weaning stage of development. Exposure extended over 38 days. The equivalent human exposure period would extend over several months.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to seriously undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Lack of detail with respect to the dams used on test (*e.g.*, number on test, weight variation, source).
- Lack of detail concerning litter numbers.
- Lack of detail concerning pup selection.
- Use of too few pups per guideline recommendations.
- Lack of description of test animal housing and husbandry.
- Use of a single exposure concentration only.
- Generally inadequate description of exposure conditions.
- Failure to include any conventional measures of toxicity as part of the study design.
- Lack of statistical analysis of the study findings.

Review – Confidence index ranking:

Low

Document Review

Author:	Larsson, KA; Eklund, AG; Hansson, L; Britt-marie, I; Malmberg, P	**Internal ID:**	662
Title:	Swine dust causes intense airways inflammation in healthy subjects.		
Year:	1994		
Abstract:	*"Fourteen healthy, nonsmoking subjects were exposed to swine dust while weighing pigs for 3 to 5 h in a swine-confinement building. All but one participant was previously unexposed to swine dust. Bronchoalveolar lavage (BAL) was performed and blood samples were drawn before and after exposure. Total dust and endotoxins were measured by air sampling in filter cassettes carried in the breathing zone. The air concentration of endotoxins was 0.6 (range, 0.08 to 1.3) $\mu g/m^3$, and of total dust was 13.5 (range, 5.6 to 24.0) mg/m^3. The air concentrations of ammonia, carbon dioxide, and hydrogen sulfide were low. The exposure induced fever in three and malaise and drowsiness in six of the subjects. Compared with preexposure values, a 75-fold increase in neutrophilic granulocytes, a two- to threefold increase in mononuclear cells, and a significant increase in eosinophilic granulocytes were observed in the BAL fluid obtained 1 day (~22 h) after exposure ($p < 0.01$ for all cell types). The fibronectin and albumin concentrations in the BAL fluid significantly increased ($p < 0.01$ for both). The number of leukocytes in peripheral blood was almost doubled 6 h after exposure and was still significantly elevated after 1 day. Blood concentrations of orosomucoid and C-reactive protein (CRP) were significantly increased 1 day after exposure. We conclude that exposure to swine dust in a swine-confinement building induces an intense inflammatory reaction in the airways as assessed by BAL. The airway cellular response is characterized by a dramatic increase in neutrophils. The components in the swine dust that cause the reaction are not clear."*		
Objective:	To analyze the cellular response and soluble markers of inflammation in brochoalveolar lavage (BAL) fluid and venous blood follwing several hours of acute exposure to swine dust during the weighing of pigs in swine confinement buildings. (Exposure to endotoxins, total dust, ammonia, carbon dioxide and hydrogen sulphide was monitored as part of the study). Exposures were monitored in healthy, previously unexposed individuals.		

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.05 ppm (whole body)	Single / exposure to "*swine dust*" lasting 2 to 5 hours	Both	Mean age 29 (Range, 21 to 51)	14. (7 men; 7 women)	Subjects were reported to be healthy	NA	NA

Observations:

Objective responses:

- Brochoalveolar lavage (BAL) analysis of cell components:
  - Total cell concentration: 4-fold increase.
  - Neutrophil concentration: 75-fold increase
  - Alveolar macrophage concentration: Doubled.
  - Lymphocyte concentration: 3-fold increase.
  - Eosinophil concentration: Increased.
  - Mast cell concentration: No change.
  - Relative numbers of alveolar macrophages, neutrophils and lymphocytes were altered following exposure

- Bronchoalveolar lavage (BAL) analysis of soluble components:
  - Albumin concentration: Increased*

  - Fibronectin concentration: Increased*
  - Hyaluronan concentration: Increased

- Venous blood analysis:
  - Leukocyte concentration: 80% increase, which did not return to baseline within 24 hours post-exposure.*
  - Orosomucoid concentration: Increased by 19% 24 hours post-exposure.*
  - C-reactive protein (CRP) concentration: Increased by 268% 24 hours post-exposure.*

- Body temperature: Increase in 3 of 14 subjects (38.2 to 39.1°C).

- IgE analysis: Positive in two of 14 subjects

- Air concentrations:
  - Dust: 13.5mg/m^3
  - Endotoxins: 0.60µg/m^3
  - Ammonia: 2 to 3ppm or 12% of the Swedish occupational exposure limit
  - $C0_2$: 0.08 to 0.1 vol.% or 20% of the Swedish occupational exposure limit.
  - H_2S: 0.05ppm or 0.5% of the Swedish occupational exposure limit.

Subjective responses:

- Headaches: Suffered by 2 of 14 subjects.
- Malaise/Drowsiness: Suffered by 6 of 14 subjects.
- Nausea: Suffered by 1 of 14 subjects.
- Airways symptoms: None reported (*i.e.*, all reactions were sub-clinical).

*Statistically significant

Author's Conclusions:

- *"Exposure to swine dust in a swine-confinement building induces an intense inflammatory reaction in the airways as assessed by BAL"*
- *"...a short exposure of healthy, previously unexposed subjects to swine dust causes a more dramatic cellular reaction in the airways than repeated, daily, exposure among swine-confinement workers."*
- *"The components of swine dust that cause the reaction are not clear."*
- *"It is not known which components in swine dust induce the airways inflammatory reaction, but endotoxins would most probably would be a contributing factor".*
- *"The concentrations of ammonia, carbon dioxide, and hydrogen sulfide found in the present study were beneath the minimal hygiene standards and were probably not important in the airways reaction that occurred on exposure to the swine-confinement buildings."*

Review – Assessment:

A. Subjects:	+ Subject profiles (gender, age, health) were adequately described. - The age range of subjects was quite large (21 to 51 years). This could increase response variability. +/- Smokers and those previously exposed to swine dust were excluded from the study.

	+ Informed consent was given. +/- Subjects had not previously been exposed to swine dust. - Number of subjects studied was limited (*i.e.*, n=14).
B. Exposure conditions:	- The exposure levels were uncontrolled. - The exposure duration was not precisely defined. Only a range was provided (*i.e.*, 2 to 5 hours). - No specifics were provided on the ventilation of the building. - Only a single point reading of the concentration of H_2S (*i.e.*, single "grab" sample) was taken. The reading was taken at the mid-exposure time using a Drager multigas detector. The location of the reading relative to the subjects was not specified. Subjects were paired, but only a single reading of H_2S concentration was taken. - Subjects were exposed to multiple agents simultaneously. This leads to difficulties is assessing cause/effect relationships and in identifying the causative agent(s). + Exposure was 'whole body', thereby mimicking exposure of the general population. - Environmental conditions (*e.g.*, humidity, temperature, airflow) were not reported.
C. Equipment:	- The concentration of H_2S was measured with a multigas detector (Drager AG).
D. Procedural:	- Blinding of subjects was not possible due to the study design. Whether technicians conducting the analyses were blinded was not noted. +/- Subjects served as their own controls, which should have limited potential confounding variables. However, the control assessment was conducted two weeks prior to exposure. Within this period, an infection or other agent may have skewed baseline values. - No follow-up exams were conducted. - Pulmonary function and bronchial responsiveness were not assessed.
E. Data collection:	- It was not possible to ascertain dose-response relationships, as there were no gradients of exposure.
F. Data analysis:	- Confidence intervals were not provided. - Raw data were not provided to permit an independent assessment of the results.
G. Interpretation:	- Lack of information pertaining to study design, limits the reproducibility of this experiment and the verification of the findings. - Exposure was to multiple agents. The authors admit that H_2S was not likely a causative factor given the very low concentrations measured.

Review - Summary:

Discussion of findings:

Exposure of previously unexposed individuals to "*swine dust*" while weighing pigs in a swine confinement building for periods up to 5 hours reportedly produced an intense inflammatory reaction of the airways, characterized most notably by an increase in the number of neutrophils in bronchiolar alveolar lavage fluid. Certain of the individuals reported subjective complaints, including headache, malaise and nausea. No symptoms of respiratory distress were reported. The components in the "*swine dust*" that caused the reaction were not clear; however, very low levels of H_2S were measured in the building.

Interpretation of the significance of the findings is undermined, in part, by lack of detail concerning actual exposure concentrations and exposure duration. In addition, the measured presence of other possible causative agents of the inflammatory response confounds any interpretation of the results and effectively precludes determination of an association between H_2S exposure and the observed airways inflammation. The other causative agents included crushed feed, grain dust, swine dander, endotoxins, lipopolysaccharides, ammonia, and total "dust".

Interpretation of the meaning and relevance of the findings should take into consideration the following:

- The concentration of H_2S measured in the building was very low (*i.e.*, 0.05 ppm). The authors admit that, given the low concentration, H_2S was "*probably not important in the airways reaction*".
- All responses affecting the respiratory system were sub-clinical in nature. Despite the cellular evidence of airway inflammation, none of the subjects reportedly experienced any airway symptoms.
- It is impossible to assign the cause of the reported airways inflammation to a single causative agent. The authors admit that: "*It is not clear what agent/agents in the swine environment are responsible for inducing airways inflammation*". However, much of the discussion of the paper centered on the contribution of endotoxins, and it was concluded that "*endotoxins would most probably be a contributing factor*".
- Total dust was highly correlated with endotoxin levels, and the increase in neutrophil levels in the lavage fluid was highly correlated with total dust, thereby possibly implicating total dust and/or endotoxins as the probable causative agent(s) of the airways reaction.
- The investigation resembled a preliminary 'case' study, identifying potential air-borne hazards associated with working in a swine confinement building. As the authors admit, further investigation would be needed to determine which components in swine dust are responsible for the airways inflammatory reaction.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions, especially as they related to the association between the airways inflammation and exposure to H_2S. Notable limitations included:

- Use of only a limited number of volunteer subjects.
- Limited and generally non-descript measurement of H_2S concentrations.
- Failure to adequately define exposure duration (*i.e.*, exposures varied from 2 to 5 hours).
- Failure to control exposure conditions (*i.e.*, exposures were performed on several different occasions).
- Failure to provide basic details concerning the swine containment building that could influence the level of exposure to any of the possible causative agents of the airways reaction (*e.g.*, size, ventilation, air exchange, temperature, animal density).
- Failure to combine the cellular measures of airway inflammation with clinical measurement of airway dysfunction (*e.g.*, spirometry).

Review – Confidence index ranking:

Low

Document Review

Author:	Kosmider, S	**Internal ID:**	694
Title:	Alkaline phosphatase activity of the blood and brain in acute experimental hydrogen sulphide intoxication.		
Year:	1967		
Abstract:	*"Alkaline phosphatase activity of blood serum and brain tissue was studied in the course of acute experimental hydrogen sulphide poisoning. It was pointed out that H_2S reduced alkaline phosphatase activity of blood serum and the brain.* *The studies of the serum of normal human subjects demonstrated that inhibition of alkaline phosphatase activity consists of conjugation of H_2S with magnesium (activator of phosphatase) to form magnesium sulphide."*		
Objective:	To investigate the effects of H_2S on the activities of serum alkaline phosphatase and brain alkaline phosphatase in rabbits following inhalation exposure. (Note that a separate investigation was performed in which the mechanism of action of H_2S on human serum alkaline phosphatase activity was examined under *in vitro* conditions. Given the nature of the investigation and the mode of exposure, *i.e., in vitro,* this portion of the study was not subject to review as part of the current assessment).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 or 71 ppm (= 0.1 mg/L) (whole body)	Single/ 1.5 hr	Rabbit	Stated to be *"mixed breed"*	1-year	Both	Total of 39 animals, consisting of a control group (n=19) and treated group (n=20).	Not specified	Not specified	71ppm

Observations:

- Clinical signs: None reported

- Alkaline phosphatase activity:
  - Serum alkaline phosphatase activity: 35% decrease*
  - Brain alkaline phosphatase activity: 35% decrease*

*Statistically significant

Author's Conclusions:

- *"It is seen from the results obtained that the acute poisoning with hydrogen sulphide causes a statistically significant decrease in the activity of serum alkaline phosphatase. A statistically significant drop in the activity of this enzyme was also observed in the brain homogenates".*
- *"Acute hydrogen sulphide intoxication causes a decrease in the activity of alkaline phosphatase of the blood and brain."*

Review – Assessment:

A. Test animals:	- The experiment was conducted on rabbits of mixed breed and sex. The distribution of sexes within each exposure group was not specified. - Pre-test health status was not indicated.

	- The breeding facility from which the animals were obtained was not noted. + The weight variation of test animals at study initiation (*i.e.*, 18%) was within recommended guidelines. + Number of test animals was adequate (*i.e.*, n=19 or 20 rabbits per group).
B. Exposure conditions:	- The source of H_2S was not given. - Only the nominal exposure concentration was reported. No indication was provided that the target concentration was analytically confirmed. - No justification was provided for selection of the exposure concentration used. - It is unclear whether the animals were placed in the exposure chamber before or after the equilibration period. This could potentially alter the duration of exposure. + The exposure period was precisely defined. + The mode of administration was whole body. - Exposure concentration, airflow, temperature and humidity were not monitored.
C. Housing/Feeding:	- Information respecting housing characteristics (materials, dimensions, bedding material, *etc.*) was lacking. - Information respecting housing conditions (*e.g.*, temperature, humidity, photoperiod) was not provided. +/- Test animals were fed a standard laboratory diet. - Feeding schedule was not specified. - Information respecting water supply was not given.
D. Equipment:	- Information pertaining to the type, dimensions, air pressure and material used in exposure device was lacking. It was described only as *"a specially constructed chamber"*. - Information respecting the gas delivery system was lacking. It was described only as *"a big Kipp apparatus"*. - No indication was provided to indicate that the target exposure concentration was analytically confirmed.
E. Procedural:	- No acclimation period or pre-test conditions (*i.e.*, quarantine, disease treatment, etc.) were specified. - It was unclear whether test animals were randomly assigned to the exposure groups. - Considering that control animals were assayed, it is unclear why alkaline phosphatase activity was determined in all treated animals 14 days before the experiment. +/- A control group of animals was included, but it is unclear whether or not controls were handled in the same manner as the treated animals. - The reason why brain homogenates were taken from the left hemisphere only was not stated. - Conventional measures of toxicity (*e.g.*, body weights, clinical observations, clinical pathology, gross pathology, organ weights) were not performed. - Only a single exposure concentration was tested. Guidelines for acute toxicity testing generally recommend testing graded dose levels. +/- Study pre-dated Good Laboratory Practice (GLP) requirements.
F. Data collection:	- Individual raw data were not provided to permit independent assessment of results.
G. Data analysis:	+/- Statistical analyses consisted of calculation of arithmetic means and standard deviations, and analysis of differences from control values using the Student's *t* test.
H. Interpretations:	- The clinical and/or toxicological significance of the changes in alkaline phosphatase activity is unknown. - No clinical symptoms were indicated despite the reductions in enzyme activities. - The dose-responsiveness of the observed changes in alkaline phosphatase activity was not established due to the use of a single exposure concentration only.

Review - Summary:

Discussion of findings:

Exposure to 71ppm H_2S for 1.5 hours reportedly caused a significant decrease in the activity of both serum and brain alkaline phosphatase in exposed rabbits. The decrease in phosphatase activity was attributed to the reaction of H_2S with magnesium, which is an activator of the enzyme.

Interpretation of the significance of the study findings should take into consideration the following:

- None of the treated animals evidently showed clinical signs of intoxication despite the inhibition of blood and brain alkaline phosphatase.
- The clinical and toxicological significance of the changes in phosphatase activity are unknown (*i.e.*, the clinical correlates of the changes are not obvious and the extent to which enzyme inhibition would need to precede any expression of clinical toxicity is unknown). The study would have been improved by including conventional measures of toxicity, such as clinical observations, body weights and gross pathology, as part of the overall study design.
- The dose-responsiveness of the observed changes could not be determined owing to the use of a single exposure concentration only. The meaning and significance of the changes might have been easier to assess had graded exposure concentrations been tested.
- The primary focus of the investigation was directed at better understanding the mechanism of H_2S at the sub-cellular level, using a metallo-enzyme suspected of being responsive to H_2S. The study was not intended to examine the health effects *per se* of H_2S.
- The inhibition of alkaline phosphatase activity was studied in isolation. Interpretation of the significance of the findings might have been eased by comparison of the responsiveness of alkaline phosphatase against the responsiveness of cytochrome oxidase, which is considered to be the primary target of H_2S.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to seriously undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Inadequate description of the exposure chamber and gas delivery system.
- Apparent lack of analytical confirmation of the target exposure concentration.
- Failure to report basic details concerning animal housing and husbandry.
- Inadequate description of exposure conditions.
- Failure to include any routine measures of toxicity as part of the study.
- Failure to include more than a single exposure concentration.

Review – Confidence index ranking:

Low

Document Review

Author:	Prior, MG; Lopez, A; Lillie, LE; Yong, S	**Internal ID:**	732
Title:	Hydrogen sulphide toxicity		
Year:	1988		
Abstract:	*"We have investigated the toxicity of hydrogen sulphide with particular emphasis on modeling human health effects. An animal model was established for studying the effects of a single exposure to hydrogen sulphide, namely young, male, Fischer-344 rats exposed to 0, 14, 279 and 558mg m^{-3} (0, 10, 200 and 400ppm) hydrogen sulphide."*		
Objective:	To examine the toxicity of hydrogen sulphide with particular emphasis on modeling human health effects. For this purpose, 4-hour exposures were performed using young male rats, with emphasis on examination of effects on cellular, enzymatic and histological markers of damage to the respiratory tree. Note that the study appears to be an extension of the work performed by Lopez *et al.* (1987, 1988 – Study ID 231 and 233).		

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0 ppm; **10** ppm; 200 ppm or 400 ppm (whole body)	Single/ Duration not specified.	Rat	Fisher-344	Not specified.(Rats were reported to be *"young"*)	Male	Unknown	Unknown	NA	NA

Observations:

- Histopathologic examination:
  - Lesions largely restricted to respiratory tract
  - Reversible necrosis of ciliated epithelium
  - Irreversible necrosis of olfactory epithelium
  - Transitory pulmonary oedema
  - Fibrinocellular alveolitis restricted to centroacinar region

- Electron microscopy
  - Reversible "blebbing" of endothelium from the basal lamina
  - The irreversible necrosis of ciliated epithelial cells was the only component of the lower airway affected.

*Statistically significant

Author's Conclusions:

- *"..lesions in acute, single exposures are largely restricted to the respiratory tract. Inhalation...resulted in severe but reversible necrosis of ciliated nasal epithelium and irreversible necrosis of olfactory epithelium. Lesions in the lung included....transitory pulmonary oedema (threshold response) and fibronocellular alveolitis"*
- *"Ciliated epithelial cells were the only component of the lower airways which suffered irreversible toxic injury...However, necrotic ciliated cells were rapidly replaced by mitosis."*

Review – Assessment:

A. Test animals:	- The number of test animals was not specified. +/- The specie, strain and sex of test animals were noted, but the precise age, health, weight variation and source of animals were omitted.
B. Exposure conditions:	- The nominal gas concentrations to which animals were exposed were stated, but there is no indication that the nominal concentrations were confirmed through sampling and analysis. - The precise duration of exposure was not defined. - The mode of administration was not recorded (*i.e.*, whole body versus nose only). - No details concerning actual exposure concentrations, airflow, temperature or humidity.
C. Housing/Feeding:	- No details respecting animal housing or husbandry were provided.
D. Equipment:	- No information pertaining to the gas delivery system, exposure chamber, or monitoring devices was provided.
E. Procedural:	- The experimental procedure was not outlined. - No indication that the study was performed under Good Laboratory Practice (GLP) conditions.
F. Data collection:	- No raw data were provided, thus limiting the independent assessment of results.
G. Data analysis:	- Details concerning statistical methods were not available.
H. Interpretations:	- Insufficient information was provided to assess the validity of the authors' conclusions. - No dose-response relationships were proposed.

Review - Summary:

Discussion of findings:

Following acute H_2S exposure, lesions of the respiratory tract evidently were shown to predominate in exposed rats. It was reported that pulmonary oedema, fibronocellular alveolitis, reversible necrosis of ciliated nasal epithelium, and irreversible necrosis of olfactory epithelium were observed upon histological examination by light and electron microscope.

Interpretation of the significance of the reported findings and conclusions is seriously undermined by the lack of detail respecting study design and conduct, as well as by reporting deficiencies. The content and descriptions found in the paper were very limited. Interpretation should take into consideration the following:

- The reported lesions of the respiratory tract of the rat following exposure to H_2S were reportedly consistent with the pulmonary oedema witnessed among individuals killed by accidental exposure to hydrogen sulphide.
- No indication of the dose-responsiveness of the lesions was indicated.
- No evidence of clinical signs of toxicity was presented. All observed effects consisted of changes at the cellular and sub-cellular level.
- The study was conducted using male rats only.

Confidence index:

Weaknesses in experimental design, conduct and/or reporting, were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Notable limitations included:

- Lack of detail with respect to number of animals on test.
- Lack of information respecting animal housing and husbandry
- Lack of detail concerning exposure conditions, exposure chambers, and the gas delivery system.

- Lack of any actual numerical data.
- Lack of description of test procedures.
- Lack of reference to any statistical analyses of the data.

Review – Confidence index ranking:

Low

APPENDIX 7

SUMMARY OF MORTALITY DATA ASSOCIATED WITH SHORT-TERM EXPOSURE TO H_2S

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure reported in reviewed documents[4] - MOUSE

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
Acute ($\leq$ 24 hours)	10 ppm	5 days	No	0 (6)	NA	Hays *et al.* (1972)	✱✱
	16 ppm	16 hours	No	0 (4)	NA	Weedon *et al.* (1940)	Study ID ◆ 316
	30 ppm	24 hours	Yes	Not specified (8)	Unknown (LT_{50} = 18.5hr)	Hays *et al.* (1972)	✱✱
	50 ppm	16 hours	Yes	Not specified (8)	Unknown (LT_{50} = 15hr)	Hays *et al.* (1972)	✱✱
	63 ppm	16 hours	Yes	4 (4)	57 minutes up to 39 hours	Weedon *et al. (1940)*	Study ID ◆ 316
	100 ppm	8 hours	Yes	3 (8)	Unknown (LT_{50} = 7.5hr)	Hays *et al.* (1972)	✱✱
		2 hours	No	0 (30)	NA	Elovaara *et al.* (1978)	Study ID ● 155
	250 ppm	16 hours	Yes	4 (4)	6.9 to 7 hours	Weedon *et al.* (1940)	Study ID ◆ 316
	1000 ppm	16 hours	Yes	4 (4)	18 to 20 minutes	Weedon *et al.* (1940)	Study ID ◆ 316
Sub-acute to sub-chronic	10 ppm	6h/d for 5d/wk for 90 days	No	0 (30)	NA	CIIT (1983)	✱
	20 ppm	48 hours	Unknown[1]	NA	NA	Hays *et al.* (1972)	✱✱
		90 days continuously	Yes[2] (?)	26 (100) NB: 16 of 100 control mice also died	Unknown	Sandage *et al.* (1961)	✱
	30 ppm	6h/d for 5d/wk for 90 days	No	0 (30)	NA	CIIT (1983)	✱
	80 ppm	6h/d for 5d/wk for 90 days	No	0 (30)	NA	CIIT (1983)	✱
	100 ppm	2h/d once every 4 days for up to 16 days	No	0 (20)	NA	Savolainen *et al.* (1980)	Study ID ● 280

✱ Study did not satisfy eligibility criteria for review since the duration of exposure extended beyond 30 days (see Chapter III: Terms of Reference). Information is included since it contributes to weight-of-evidence.

✱✱ Study is not a full-length peer-reviewed document, and thus, did not satisfy the eligibility criteria for review (see Chapter III: Terms of Reference). Information is included since it contributes to weight-of-evidence.

✱✱✱ Reproductive and/or developmental toxicity study. Although duration of exposure extended beyond 30 days, study was included as part of the dataset and was subjected to detailed review.

[1] Author(s) made no explicit statement with respect to whether or not any animals died on test. Given the obvious and frank nature of the response and it seriousness, it is expected that had treatment-related deaths occurred, they would have been reported. However, some uncertainty remains.

[2] The study investigators indicated that deaths could not be entirely ascribed to treatment since a significant number of control animals (*i.e.*, 0 ppm H_2S) also died on test.

[3] Duration of exposure was not well specified.

[4] Only the mortality findings from reviewed documents (*i.e.*, those satisfying the eligibility criteria specified in Chapter III: Terms of Reference) are summarized. Additional findings from studies that did not satisfy the eligibility criteria, but which used exposure concentrations within the range of interest, were readily available, and/or are often cited in the published literature have been included since they contribute to the weight-of-evidence. However, this additional set of studies should not be considered all-inclusive.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] – RAT

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
Acute (≤ 24 hours)	10 ppm	3 hours	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		4 hours	No	0 (4 to 6)	NA	Khan *et al.* (1990)	Study ID ◆ 210
		4 hours	No	0 (12)	NA	Lopez *et al.* (1987)	Study ID ◆ 231
		4 hours	Unknown[1]	NA	NA	Lopez *et al.* (1988)	Study ID ◆ 233
		Duration not well specified	Unknown[1]	NA	NA	Prior *et al.* (1988)	Study ID ● 732
	16 ppm	16 hours	No	0 (8)	NA	Weedon *et al* (1940)	Study ID ◆ 316
	30 ppm	3 hours	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
	40 ppm	6 hours	No	0 (12)	NA	Lopez *et al.* (1986)	Study ID ◆ 466
	45 ppm	2 to 6 hours	Unknown[1]	NA	NA	Rogers and Ferin (1981)	Study ID ◆ 269
	50 ppm	4 hours	No	0 (4 to 6)	NA	Khan *et al.* (1990)	Study ID ◆ 210
		4 hours	Unknown[1]	NA	NA	Khan *et al.* (1991)	Study ID ◆ 211
	63 ppm	16 hours	Yes	1 (8)	During 16 hours exposure	Weedon *et al.* (1940)	Study ID ◆ 316
	80 ppm	3 hours	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
	83 ppm	4 hours	Unknown[1]	NA	NA	Lopez *et al.* (1988)	Study ID ◆ 232
	190 to 240 ppm	Up to 18 hours[3]	Yes	Not specified (17)	8 to 18 hours	Mitchell and Yant (1925)	Study ID ● 444
	200 ppm	3 hours	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		4 hours	No	0 (4 to 6)	NA	Khan *et al.* (1990)	Study ID ◆ 210
		4 hours	Unknown[1]	NA	NA	Khan *et al.* (1991)	Study ID ◆ 211
		4 hours	No	0 (12)	NA	Lopez *et al.* (1987)	Study ID ◆ 231
		4 hours	Unknown[1]	NA	NA	Lopez *et al.* (1988)	Study ID ◆ 233
		Duration not well specified	Unknown[1]	NA	NA	Prior *et al.* (1988)	Study ID ● 732
	250 ppm	16 hours	Yes	8 (8)	17.9 to 22.9 hours	Weedon *et al.* (1940)	Study ID ◆ 316
	300 ppm	6 hours	Yes	12 (12)	Within the 6 hour exposure period	Lopez *et al.* (1986)	Study ID ◆ 466
	310 to 350 ppm	Up to 8 hours[3]	Yes	Not specified (13)	1 to 8 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	400 ppm	3 hours	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		4 hours	No	0 (4 to 6)	NA	Khan *et al.* (1990)	Study ID ◆ 210
		4 hours	Unknown[1]	NA	NA	Khan et al. (1991)	Study ID ◆ 211
		4 hours	No	0 (12)	NA	Lopez *et al.* (1987)	Study ID ◆ 231
		4 hours	Unknown[1]	NA	NA	Lopez *et al.* (1988)	Study ID ◆ 233
		Duration not well specified	Unknown[1]	NA	NA	Prior *et al* (1988)	Study ID ● 732
	439 ppm	4 hours	Unknown[1]	NA	NA	Lopez *et al.* (1988)	Study ID ◆ 232
	450 ppm	Up to 4 hours[3]	Yes	Not specified (2)	1 to 4 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] – RAT

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
	520 to 530 ppm	Up to 4 hours[3]	Yes	Not specified (3)	1 to 4 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	620 ppm	Up to 1 hour[3]	Yes	Not specified (3)	30 minutes to 1 hour into exposure	Mitchell and Yant (1925)	Study ID ● 444
	500 to 700 ppm	4 hours	Yes	4 to 6 (4 to 6)	Within exposure period.	Khan *et al.* (1990)	Study ID ◆ 210
	790 to 900 ppm	Up to 1 hour[3]	Yes	Not specified (40)	2 minutes to 1 hour into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1000 ppm	37 minutes	Yes	8 (8)	29 to 37 minutes	Weedon *et al.* (1940)	Study ID ◆ 316
Sub-acute to sub-chronic	7.14 ppm	Unknown number of hours per day, from the first to twentieth day of pregnancy.	Unknown[1]	NA	NA	Barilyak and Vasil'eva (1974)	Study ID ● 497
	10 ppm	6h/d for 5d/wk for 90 days	No	0 (60)	NA	CIIT (1983)	*
		6h/d for 60 days	No	0 (24)	NA	Dorman *et al.* (2000)	Study ID ▲ 149/594 ***
		3h/d for 5 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		6h/d for 70 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151 ***
		3h/d for 5 days	No	0 (20)	NA	Struve *et al.* (2001)	Study ID ◆ 296
	20 ppm	90 days continuously	Yes	12 (50)	Unknown	Sandage *et al* (1961)	*
		7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Hannah and Roth (1991)	Study ID ● 11 ***
		7h/d from day 6 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al.* (1990)	Study ID ◆ 12 ***
		7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Skrajny *et al.* (1992)	Study ID ◆ 290 ***
		7h/d from day 1 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al.* (1990)	Study ID ◆ 564 ***
	25 ppm	3h/d for 5 consecutive days	Unknown[1]	NA	NA	Skrajny *et al.* (1996)	Study ID ◆ 291
	30 ppm	6h/d for 5d/wk for 90 days	No	0 (60)	NA	CIIT (1983)	*
		6h/d for 60 days	No	0 (24)	NA	Dorman *et al.* (2000)	Study ID ▲ 149/594 ***
		3h/d for 5 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		6h/d for 70 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151 ***
		3h/d for 5 days	No	0 (20)	NA	Struve *et al.* (2001)	Study ID ◆ 296

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] – RAT

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
	35 to 65 ppm	Up to 100 hours[3]	No	0 (4)	NA	Mitchell and Yant (1925)	Study ID ● 444
	50 ppm	7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Hannah and Roth (1991)	Study ID ● 11 ***
		7h/d from day 6 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al* (1990)	Study ID ◆ 12 ***
		6h/d from day 6 to 20 of gestation	Unknown[1]	NA	NA	Saillenfait *et al* (1989)	Study ID ◆ 278
		3h/d for 5 days	Unknown[1]	NA	NA	Skrajny *et al.* (1996)	Study ID ◆ 291
		7h/d from day 6 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Hannah *et al* (1989)	Study ID ● 402 ***
		7h/d from day 1 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al.* (1990)	Study ID ◆ 564 ***
	75 ppm	7h/d from day 6 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al.* (1990)	Study ID ◆ 12 ***
		7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Hannah *et al.* (1989)	Study ID ● 180 ***
		7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Skrajny *et al.* (1992)	Study ID ◆ 290 ***
		3h/d for 5 days	Unknown[1]	NA	NA	Skrajny *et al.* (1996)	Study ID ◆ 291
		7h/d from day 1 of gestation until day 21 postpartum	Unknown[1]	NA	NA	Hayden *et al.* (1990)	Study ID ◆ 564 ***
		7h/d from day 5 postcoitus until day 21 postnatal	Unknown[1]	NA	NA	Roth and Hannah (1989)	Study ID ● 630 ***
	80 ppm	6h/d for 5d/wk for 90 days	No	0 (60)	NA	CIIT (1983)	*
		6h/d for 60 days	No	0 (24)	NA	Dorman *et al.* (2000)	Study ID ▲ 149/594 ***
		3h/d for 5 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		6h/d for 70 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151 ***
		3h/d for 5 days	No	0 (20)	NA	Struve *et al.* (2001)	Study ID ◆ 296
	100 ppm	6h/d from day 6 to 20 of gestation	Unknown[1]	NA	NA	Saillenfait *et al* (1989)	Study ID ◆ 278
		3h/d for 5 days	Unknown[1]	NA	NA	Skrajny *et al* (1996)	Study ID ◆ 291
		3h/d for 5 days	Unknown[1]	NA	NA	Nicholson *et al* (1998)	Study ID ◆ 398
	100 to 140 ppm	Up to 48 hours[3]	Yes	Not specified (19)	18 to 48 hours	Mitchell and Yant (1925)	Study ID ● 444

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] – RAT

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
	150 ppm	6h/d from day 6 to 20 of gestation	Unknown[1]	NA	NA	Saillenfait *et al* (1989)	Study ID ◆ 278
	200 ppm	3h/d for 5 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		3h/d for 5 days	No	0 (10)	NA	Struve *et al.* (2001)	Study ID ◆ 296
	400 ppm	3h/d for 5 days	Unknown[1]	NA	NA	Dorman *et al.* (2002)	Study ID ◆ 151
		3h/d for 5 days	Yes	1 (10)	Unknown	Struve *et al.* (2001)	Study ID ◆ 296

* Study did not satisfy eligibility criteria for review since the duration of exposure extended beyond 30 days (see Chapter III: Terms of Reference). Information is included since it contributes to weight-of-evidence.

** Study is not a full-length peer-reviewed document, and thus, did not satisfy the eligibility criteria for review (see Chapter III: Terms of Reference). Information is included since it contributes to weight-of-evidence.

*** Reproductive and/or developmental toxicity study. Although duration of exposure extended beyond 30 days, study was included as part of the dataset and was subjected to detailed review.

[1] Author(s) made no explicit statement with respect to whether or not any animals died on test. Given the obvious and frank nature of the response and it seriousness, it is expected that had treatment-related deaths occurred, they would have been reported. However, some uncertainty remains.

[2] The study investigators indicated that deaths could not be entirely ascribed to treatment since a significant number of control animals (*i.e.*, 0 ppm H_2S) also died on test.

[3] Duration of exposure was not well specified.

[4] Only the mortality findings from reviewed documents (*i.e.*, those satisfying the eligibility criteria specified in Chapter III: Terms of Reference) are summarized. Additional findings from studies that did not satisfy the eligibility criteria, but which used exposure concentrations within the range of interest, were readily available, and/or are often cited in the published literature have been included since they contribute to the weight-of-evidence. However, this additional set of studies should not be considered all-inclusive.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] - OTHER SPECIES

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
CANARY							
Acute (≤ 24 hours)	35 to 65 ppm	Up to 18 hours[3]	Yes	Not specified (2)	8 to 18 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	97 to 100 ppm	Up to 8 hours[3]	Yes	6 (6)	4 hours into exposure up to 12 to 36 hours following exposure	Mitchell and Yant (1925)	Study ID ● 444
	140 ppm	Up to 8 hours[3]	Yes	4 (4)	4 hours into exposure up to 12 to 36 hours following exposure	Mitchell and Yant (1925)	Study ID ● 444
	190 to 210 ppm	Up to 1 hour[3]	Yes	Not specified (4)	Following 30 minutes of exposure	Mitchell and Yant (1925)	Study ID ● 444
	280 to 310 ppm	Up to 30 min[3]	Yes	Not specified (3)	Following 2 minutes of exposure	Mitchell and Yant (1925)	Study ID ● 444
	440 ppm	Up to 2 min[3]	No	0 (4)	NA	Mitchell and Yant (1925)	Study ID ● 444
	520 ppm	Up to 2 min[3]	No	0 (1)	NA	Mitchell and Yant (1925)	Study ID ● 444
	620 ppm	Up to 2 min[3]	No	0 (2)	NA	Mitchell and Yant (1925)	Study ID ● 444
	730 ppm	Up to 2 min[3]	Yes	Not specified (Unknown)	18 to 20 sec	Mitchell and Yant (1925)	Study ID ● 444
DOG							
Acute (≤ 24 hours)	103 ppm	Up to 16 hours[3]	Yes	Not specified (2)	8 to 16 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	240 ppm	Up to 16 hours[3]	Yes	2 (2)	8 to 16 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	350 ppm	Up to 16 hours[3]	Yes	2 (2)	4 to 16 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	760 to 800 ppm	Up to 1 hour[3]	Yes	1 (2)	30 minutes to 1 hour into exposure	Mitchell and Yant (1925)	Study ID ● 444
	850 to 890 ppm	Up to 30 min[3]	Yes	Not specified (3)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1000 to 1140 ppm	Up to 30 min[3]	Yes	Not specified (8)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1280 ppm	Up to 30 min[3]	Yes	Not specified (4)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1500 to 1600 ppm	Up to 30 min[3]	Yes	Not specified (9)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] - OTHER SPECIES

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
GOAT							
Acute (≤ 24 hours)	10 ppm	4 days continuously	Unknown[1]	NA	NA	Hays *et al.* (1972)	**
	50 ppm	4 days continuously	Unknown[1]	NA	NA	Hays *et al.* (1972)	**
	100 ppm	4 days continuously	Unknown[1]	NA	NA	Hays *et al.* (1972)	**
	820 ppm	Up to 30 min[3]	No	0 (1)	NA	Mitchell and Yant (1925)	Study ID ● 444
	1000 to 1100 ppm	Up to 30 min[3]	Yes	Not specified (4)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1280 to1330 ppm	Up to 30 min[3]	Yes	Not specified (4)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
GUINEA PIG							
Acute (≤ 24 hours)	100 ppm	1 hour	Unknown[1]	NA	NA	Hulbert *et al* (1989)	Study ID ● 460
	240 ppm	Up to 18 hours[3]	Yes	2 (3)	8 to 18 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	500 ppm	1 hour	Unknown[1]	NA	NA	Hulbert *et al. (1989)*	Study ID ● 460
	820 ppm	Up to 48 hours[3]	No	0 (5)	NA	Mitchell and Yant (1925)	Study ID ● 444
	1000 to 1100 ppm	Up to 30 min[3]	Yes	Not specified (10)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
	1500 ppm	Up to 30 min[3]	Yes	Not specified (2)	2 to 30 minutes into exposure	Mitchell and Yant (1925)	Study ID ● 444
Sub-acute to sub-chronic	20 ppm	1h/d for 11 days	Unknown[1]	NA	NA	Haider *et al.* (1980)	Study ID ● 179
	35 to 65 ppm	Up to 100 hours[3]	No	0 (2)	NA	Mitchell and Yant (1925)	Study ID ● 444
	103 ppm	Up to 48 hours[3]	Yes	1 (2)	18 to 48 hours into exposure	Mitchell and Yant (1925)	Study ID ● 444
	350 ppm	Up to 54 hours[3]	Yes	3 (3)	8 hours into exposure up to 4 days following exposure	Mitchell and Yant (1925)	Study ID ● 444

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

Summary of mortality data associated with H_2S exposure[4] - OTHER SPECIES

Exposure classification	Exposure concentration	Exposure duration	Deaths?	Number of deaths (Number of animals exposed)	Time until death	Author(s)	Study ID
MONKEY							
Acute (≤ 24 hours)	20 ppm	90 days continuously	No	0 (10)	NA	Sandage *et al.* (1961)	*
PIG							
Acute (≤ 24 hours)	8.5 ppm	17 days	Unknown[1]	NA	NA	Curtis *et al* (1975)	Study ID ● 416
	50 to 100 ppm	2 hours	Unknown[1]	NA	NA	O'Donoghue (1961)	Study ID ● 255
	400 ppm	1 sec	Yes	1 (1)	1 sec	O'Donoghue (1961)	Study ID ● 255
	250 to 970 ppm	230 minutes	No	0 (1)	NA	O'Donoghue (1961)	Study ID ● 255
	250 to 1000 ppm	130 minutes	Yes	1 (1)	130 minutes	O'Donoghue (1961)	Study ID ● 255
	350 to 1200 ppm	44 minutes	Yes	1 (1)	44 minutes	O'Donoghue (1961)	Study ID ● 255
	500 to 1050 ppm	36 minutes	No	0 (1)	NA	O'Donoghue (1961)	Study ID ● 255
RABBIT							
Acute (≤ 24 hours)	50 ppm	16 hours	No	0 (3)	NA	O'Donoghue (1961)	Study ID ● 255
	71 ppm	1.5 hours	Unknown[1]	NA	NA	Kosmider *et al* (1967)	Study ID ● 223
		1.5 hours	Unknown[1]	NA	NA	Kosmider (1967)	Study ID ● 694
	1000 ppm	Accidental exposure	Yes	1 (3)	2 hours following exposure	O'Donoghue (1961)	Study ID ● 255
Sub-acute to sub-chronic	71 ppm	30 min/d for 5 days	Unknown[1]	NA	NA	Kosmider *et al* (1967)	Study ID ● 223
		1h/d for 14 days	Unknown[1]	NA	NA	Kosmider *et al* (1971)	Study ID ● 595

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